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Journal of the Fisheries Research Board of Canada

Analysis of Laboratory Experiments on Growth Efficiency of Fishes[1,2]

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Paloheimo and Dickie (J. Fish. Res. Bd. Canada 23: 869–908, 1966) described a regular variation between growth efficiency and ration level of fishes, termed a "K-line." Subsequent work has either confirmed the phenomenon, or suggested that it is a special case observable only at high ration levels. Two recent published experiments are reanalyzed with the aid of an analytic model based upon the metabolic requirements of fishes. The reality of the K-line is confirmed, and it is concluded that it occurs because metabolic expenditure upon spontaneous activity increases more than proportionately to increased rations, increasing the fraction of ration energy dissipated as metabolism, and thereby decreasing growth efficiency.

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ACCURATE prediction of fish growth is essential to intelligent management of any fishery. Of the growth models generally in use, the most satisfactory, and heuristic, are based upon biologically meaningful functions. Thus, the widely used Bertalanffy equation derives from the a priori logic of the balance between anabolism and catabolism (see Allen 1969). More recently, Paloheimo and Dickie (1965) extended the reductionist approach to growth, with a model based primarily upon an empirical relation between rations and growth efficiency, termed a "K-line." The advantage of the latter approach consists in its directly observable, rather than conceptual, basis; its disadvantage consists in the difficulty in resolving the properties of K-lines, as shown by contradictory evidence in the subsequent literature.

In adopting the reductionist approach to growth, the problem becomes one of comprehending the processes reflected in growth efficiency, K. Analysis of the problem falls naturally into three parts: laboratory analysis of K, prediction of K in nature, and application of K to growth prediction (Kerr 1971a, b). In the present paper, I attempt to elucidate the K-line phenomenon as observed in the laboratory, by providing a mechanistic explanation that reconciles otherwise contradictory evidence.

[1]The second of a series of four papers based on a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy from Dalhousie University, Halifax, N.S.

[2]B.I. Contribution No. 241.

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The K-Line Phenomenon

Paloheimo and Dickie (1966b), reanalyzing laboratory food and growth experiments, found evidence of regular variation of gross growth efficiency (the fraction of rations appearing as growth) with changes in level of rations or size of predator. In many instances, the regular variation in growth efficiency could be satisfactorily described by a straight line, a K-line, of zero or negative slope, if growth efficiency were plotted on a logarithmic ordinate.

Their extensive examination (Paloheimo, and Dickie 1966a, b) of the interactions among food, temperature, body size, metabolism, and growth, clearly showed the complexity of the system underlying measures of growth efficiency. Nevertheless, certain further regularities were observed. Thus, comparisons among experiments indicated that shallower K-line slopes resulted with larger food particles. Accordingly, they suggested that the variable energy costs inherent in foraging for food items of differing relative size might in part determine growth efficiency. Reasoning from field evidence that "... fish flesh would afford a much more substantial diet per unit of effort than plankton...," Martin (1952) reached a similar conclusion, attributing the more rapid growth of piscivorous lake trout to enhanced foraging efficiency.

It appears, however, that a fundamental distinction must be made between laboratory and field determinations of growth efficiency. In the laboratory, a single fish or minor population is confined within a small space and provided with a prescribed level of rations. In nature, the acquired ration depends upon a complex array of biotic and abiotic parameters, not least of which is a foraging or search requirement often lacking in the laboratory environment. The attendant constraints are best analyzed separately.

Analysis of Growth Efficiency in the Laboratory

In this section, I develop a simple analytic model of the growth process in the laboratory, primarily as a framework for subsequent discussion of laboratory evidence. For clarity, I have based my model upon a specific predator, the brook trout (*Salvelinus fontinalis*).

Conventions

Certain conventions are both necessary and have been widely applied to analysis of the energy relations of fishes. I now briefly detail those that I have applied here.

For conversion of energy units I have assumed that 1 liter of respired oxygen is equivalent to 4.8 kcal, that 1 g wet weight of tissue yields 1 kcal, and that the energy content of the predator is equivalent to that of the same weight of prey. Further, I have usually assumed that fish normally assimilate 80% of the ingested ration, giving an assimilation coefficient, p, equal to 0.8. These choices have been repeatedly discussed, in particular by Winberg (1956) and Paloheimo and Dickie (1966a).

To describe the relation between body weight and metabolism of fish, I use here the familiar

$$T = \alpha W^{\gamma}, \tag{1}$$

where T indicates total metabolism, and α and γ are fitted parameters. The applicability of equation 1 to the metabolism of fishes has been thoroughly examined by Winberg (1956) and Paloheimo and Dickie (1966a) who conclude that for most species γ is approximately 0.8 and remains relatively stable under a variety of temperature regimes and metabolic loads. Neither Brett (1965) nor Job (1955) found γ to be completely stable for sockeye salmon (*Oncorhynchus nerka*) or *S. fontinalis* respectively, when exercised beyond the standard level, but the observed values were nevertheless uniformly less than unity. Beamish (1964), also working with brook trout, found values of γ for standard metabolism that were not significantly different from unity, but neither were these values statistically distinct from the more extensive data of Job. As a standard, I have elected $\gamma = 0.85$ in subsequent analysis, although any value of γ less than unity would not affect my conclusions.

The level of metabolism, α, can assume values between the standard and active levels. Evaluation of studies of brook trout by Graham (1949), Job (1955), and Beamish (1964) indicates good agreement at 13 C, where $\alpha_S = 0.015$ provides a good estimate of the level of standard metabolism, in units of kcal/day. Similar comparisons among work by Basu (1959), Graham (1949), and Job (1955) again indicate substantial agreement at 13 C, yielding a value of $\alpha_A = 0.066$ for the level of active metabolism. All rates in this study are based on a temperature of 13 C, a level that may be considered "optimum" for brook trout, in the sense that Baldwin (1956) observed maximum levels of feeding and growth at that temperature.

The Model

The total metabolism, T_T, expended per day may be regarded as consisting of several elements: T_F, the cost of foraging activity; T_S, the cost of standard metabolism; T_C, the energy cost specific to utilization of food; and T_R, the energy expended upon spontaneous activity; i.e.,

$$T_T = T_F + T_S + T_C + T_R. \tag{2}$$

In the laboratory, the term T_F will often be trivial; space limitations and the usual feeding protocols clearly obviate any great expenditure of energy upon search

and capture of rations, except in the few instances (e.g., Baldwin 1956) where capably fugitive, living prey have been offered. Moreover, the T_F component will be proportional to the number of prey taken, and can therefore be described by

$$T_F = Z\ (R/g), \tag{3}$$

where R is the ration, Z is the average energy cost of acquiring a single diet item, and g is the weight of an individual diet item. Although Z can be expected to vary in complex fashion with predator size, prey mobility, and the like, within any given experimental conditions it will normally exert a relatively constant effect.

T_C, the energy cost specific to utilization of food, is likely to be important at any significant level of feeding. As a rule, the internal efficiency of converting metabolizable food to body substance is 60–70%, based upon the efficiency of energy transfer at the biochemical level (Krebs and Kornberg 1957).

The calorigenic metabolism, sometimes referred to as the specific dynamic action, is a measure of the metabolic response to ingestion of food, and represents the major portion of the metabolic cost of converting food to body substance. Barring unusual types of diet, it is usually found that the calorigenic metabolism is proportional to the ingested ration, and independent of body weight (Brody 1945; Kleiber 1961).

The metabolic cost, T_C, of converting food to new body substance is most easily determined from feeding experiments. Several sources (Baldwin 1956; Phillips et al. 1940; Tunison et al. 1939; Surber 1935) provide information suitable for determination of the relation between ration level and T_C for the brook trout.

The total metabolic expenditure, T_T, may be determined from the balanced energy equation (Winberg 1956) by subtraction:

$$pR = T_T + \Delta W, \tag{4}$$

where R is the ration, corrected by p for nonassimilation, and ΔW the growth increment. That is, I found the total metabolic expenditure for each sample period by subtracting the energy equivalent of growth from that of the assimilated rations. From the difference, I then subtracted the standard metabolism, which was computed for each fish size and experimental temperature by using relations derived from pooled data of Graham (1949), Job (1955), and Beamish (1964). As derived, the resulting values undoubtedly incorporate energy losses due to spontaneous external activities. That is, the values provide a combined measure of $T_C + T_R$.

Spontaneous activity of brook trout is known to vary markedly with temperature (Fisher and Sullivan 1958). Beamish (1964) has determined the "average routine scope for activity," the fraction of metabolism spontaneously expended by brook trout held at various temperatures. Although his fish were not fed during or immediately prior to testing, the routine scope was determined for trout accustomed to being fed all they would eat once daily (F. W. H. Beamish personal communication). Paloheimo and Dickie (1966a) have shown that this level of ration will induce an intermediate metabolic plane somewhat less than the maximum level elicited by an ad libitum feeding regime. In large part, the trout in the four feeding experiments I have reanalyzed have been shown (Paloheimo and Dickie 1966a) to have existed on a single, approximately maximum metabolic plane corresponding to an ad libitum feeding regime. To ensure that uniformly high metabolic planes were used throughout, I have discarded the few data observed at other metabolic levels. For each fish considered in my analysis I then computed a routine scope corresponding to the relation described by Beamish. As the routine scope constitutes a measure of the cost of spontaneous activity for fish on the specified nutritional plane, I was thus able to correct my combined measures of $T_C + T_R$ by subtracting the routine scope estimates, deriving a measure of T_C for a wide range of rations. The relation between T_C and rations (Fig. 1) is clearly linear, and is adequately described by

$$T_C = mR \tag{5}$$

where the slope m equals 0.2882. The value of m is within the range corresponding to a 60–70% conversion efficiency, and the linearity of equation 5 is also as expected.

The response of T_R to changing levels of feeding is relatively unknown. As the subject will be further discussed below, it is sufficient to note here that the consistent use of uniform nutritional planes in deriving my estimates of T_C obviates errors deriving from uncertainty about the precise form of the T_R-R relation within experiments. Accordingly, I have taken equation 5 as a fair representation of the calorigenic metabolism normally induced in brook by varying levels of ration.

Substituting for T_F and T_C from equations 3 and 5 respectively, equation 2 may now be rewritten as

$$T_T = Z(R/g) + mR + T_S + T_R. \tag{6}$$

Simultaneously substituting from equation (1) for the various T components, equation 6 can be solved for R.

$$R = \frac{g(\alpha_T - \alpha_S - \alpha_R)W^{\gamma}}{Z + gm}. \tag{7}$$

Division of equation 4 by R provides an expression of growth efficiency,

$$K = \Delta W/R = p - T_T/R,$$

which can be further expanded, by substituting for T from equation 1 and R from equation 7, to the form

$$K = p - \frac{\alpha_T(Z + gm)}{g(\alpha_T - \alpha_R - \alpha_S)}. \tag{8}$$

As an aid to assessment, it is helpful to substitute the numerical values derived for the brook trout into equations 7 and 8:

$$R = \frac{g(\alpha_T - \alpha_R - 0.015)W^{0.85}}{Z + 0.2882\ g}, \tag{7}$$

$$K = 0.8 - \frac{\alpha_T(Z + 0.2882\, g)}{g(\alpha_T - \alpha_R - 0.015)}, \qquad (8)$$

where $\alpha_T \leqslant \alpha_A = 0.066$, and $\alpha_R \leqslant \alpha_T - \alpha_S \leqslant \alpha_A - \alpha_S = 0.051$.

Several biologically plausible mechanisms capable of determining variations in growth efficiency can be inferred from the forms of equations 7 and 8. I shall note these here, but assess them subsequently in the light of recent empirical evidence.

First, equation 7 indicates that the level of total metabolism, α_T, will be proportional to the assigned level of rations. This property is simply the converse of Warren and Davis' (1967) concept of "scope for growth," which in turn is a restatement of Fry's (1957) "scope for activity." Alternatively, where body weight is the sole experimental variable, metabolic level will vary inversely as W^{γ}. If Z, the average cost of a single diet item, is itself appreciable, then g, the weight of a diet item, can have a significant effect upon the equation. For the present, it is sufficient to assume that Z is relatively small in most aquarium experiments, and consequently, that neither Z nor g exert significant effects on equations 7 or 8. This assumption will be relaxed subsequently.

Second, equation 8 indicates that K would show progressive decrease should p, the assimilation coefficient, decrease with greater rations. The available evidence indicates that p may indeed decrease significantly at high levels of ration, a relation that has been suggested elsewhere (Warren and Davis 1967; Brett et al. 1969) as an explanation of the K-line phenomenon. I do not accept this explanation. If smaller fractions of large rations are assimilated, then the metabolic cost of processing the ration must also decrease proportionately, counteracting the tendency to decrease K. In terms of the equation system, this effect is apparent if equation 5 is derived as a function of the assimilated, rather than the total ration, leading to the form

$$T_C = m'pR. \qquad (5a)$$

If equation 8 is now derived, as before but using 5*a* rather than 5, we obtain

$$K = p - \frac{\alpha_T(Z + gm'p)}{g(\alpha_T - \alpha_R - \alpha_S)}. \qquad (8a)$$

From equation 8*a*, any change in p will not greatly affect the value of K, in most biologically plausible circumstances. Accordingly, I have rejected the effect of the assimilation coefficient as an important determinant of growth efficiency.

Also with respect to equation 8, there is no reason to suppose that α_R is invariate under different feeding regimes; if α_R should increase more rapidly than α_T, K must decrease.

Finally, it should be noted that equations 7 and 8 must be applied with appropriate consideration of the properties of their components. Thus, whereas equation 8 appears independent of both R and W, the former is correlated with α_T, and Z may depend on W. Nevertheless, the equations provide a convenient frame for evaluation of laboratory studies of growth efficiency.

In reality, effects of changes in a single variable cannot be isolated in growth experiments. Any experimental investigation of growth efficiency must, as a minimum, measure the combined effects of changes in both body size and level of rations. In fact, most published evidence derives from complex experimental designs and analyses in which the mechanisms suggested above have been combined to varying degrees. It is not surprising, therefore, that contradictory conclusions have been reached by various investigators.

Evaluation of Recent Evidence

Two recent papers provide new information on the K-line phenomenon. Both warrant careful consideration. Brett et al. (1969), working with *Oncorhynchus nerka*, have concluded that the K-line phenomenon is a special instance, observable only at high ration levels. In contrast, LeBrasseur (1969) obtained evident K-lines with *O. keta* grown over a wide range of feeding levels. As these views appear mutually exclusive, it is useful to consider each paper in turn.

LeBrasseur's Chum Salmon

LeBrasseur's fig. 1 indicates that at low ration levels his salmon had growth efficiencies somewhat in excess of unity, provoking a theoretical dilemma that is resolved by examination of his methods. LeBrasseur pumped sea water to his tanks through a coarse-mesh intake screen, and estimated that 40–70 microzooplankton less than 2 mm in diameter passed the screen per hour. An illicit food input would most affect the apparent growth efficiency of fish held at low ration levels, artificially elevating the K estimates. If it is assumed that the illicit food particles averaged 2.5 mg in wet weight, an additional 185 g of food organisms would have entered the tanks during an 8-week experiment. That is, the actual feeding levels may have ranged from 200 to 122% of the nominal lowest and highest rates respectively. Although this assumption is almost certainly excessive, in the following reanalysis of LeBrasseur's data I have preferred to err on the conservative side, eliminating any chance of spurious K-lines resulting from the greater effect of illicit rations at low experimental rations.

LeBrasseur assigned rations in fixed proportion to body weight. That is, the ration available to each individual was not constant, but increased progressively throughout the course of the experiment. It has been shown by Paloheimo and Dickie (1966a), and it is also clear from equation 7, that as the fish grow, a proportional feeding

regime will result in a continuous increase in metabolic level within the permissible biokinetic range. Using equation 4, I have confirmed this trend in LeBrasseur's data (Fig. 2). The decreasing slopes in Fig. 2 indicate progressive increase of metabolic level with level of ration, up to the maximum limit. It is clear, therefore, that at least three important parameters, rations, body weight, and level of metabolism, varied throughout the experiment.

Although the level of metabolism is therefore confounded with body weight, the effect can be essentially eliminated by stratifying the weekly observations within weight classes. I have found that 1-g intervals are adequate. My estimates of K, adjusted for illicit rations and rejecting the few data that are not closely described by LeBrasseur's fitted growth curves, are plotted for the several weight groupings against the level of rations in Fig. 3. The resulting lines provide clear confirmation that LeBrasseur has observed the K-line phenomenon over relatively wide ranges of ration. Although only a portion of the reanalysis is illustrated in Fig. 3, it should be noted that the remaining data are quite comparable. Similarly, the few data rejected for reasons given above also correspond to the pattern illustrated. It can also be noted that doubling the assumed input of illicit ration, or eliminating it completely, affects only the relative scale of the results, and does not eliminate the clear negative regressions of log K against R.

The slopes of the several K-lines in Fig. 3 decrease progressively with increasing fish size. I have shown that body weight is confounded with metabolic level because the data for larger fish were derived at increasingly higher and narrower ranges of metabolism (Fig. 2). Accordingly, the decreasing slopes reflect decreasing metabolic ranges.

It remains to enquire why K should have decreased in these experiments with increasing level of metabolism. Aside from any theoretical considerations on this point, the correlation is observational fact; its explanation promises to account for the K-line phenomenon.

According to equation 8, if α_S and α_R remain constant, an increased α_T must induce a higher level of K. There is no reason to suppose, however, that α_R would remain constant with changing levels of R; indeed, the available laboratory evidence suggests that α_R will increase more than proportionately to increasing α_T or R.

Brown (1957) concluded on the basis of earlier work (Brown 1946) with brown trout that spontaneous activity varied with feeding level. In the course of determining maintenance rations, she observed that a ration that initially elicited a weight loss was capable of supporting growth after a short period of adjustment. She found her ob-

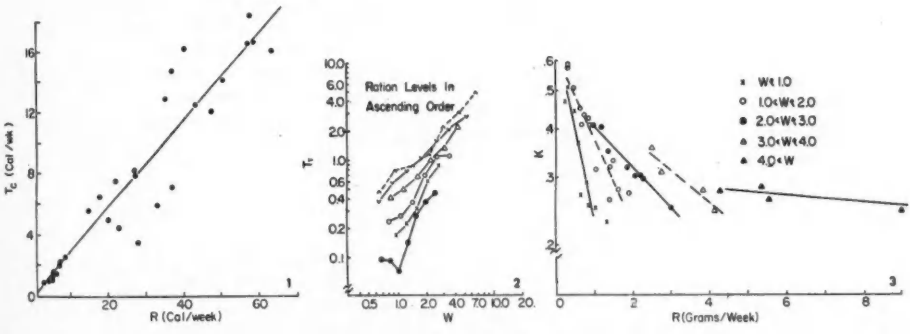

FIG. 1. The metabolic expenditure specific to the conversion of rations to body substance, T_C, expressed as a function of ration level, R. The fitted straight line (least squares) is described by $T_C = 0.2882R$.

FIG. 2. The relation of total metabolism to body weight for different feeding levels (i.e., where rations are a constant proportion of body weight). The level of metabolism rises, and the slope of the relation decreases, as ration level increases, indicating that metabolic level increased (within the biokinetic range) as the fish grew on a constant feeding level. Calculations are based on data of LeBrasseur (1969). Ration levels in ascending order are indicated as ●, ×, ○, △, V, Λ.

FIG. 3. Relations of growth efficiency, K, to ration level, i.e., K-lines, for salmon at 1-g intervals of body size. The decreasing slopes with increasing size of fish reflect decreasing ranges of metabolic level, as can be seen by comparison with Fig. 2. Calculations based on data of LeBrasseur (1969).

servations paralleled a demonstration by Fry (1957) of a relation between temperature and spontaneous activity, and concluded that her observations reflected reduced activity in response to reduced rations.

Precise data bearing upon the level of activity induced by specific levels of ration are not abundant. Magnuson (1970) reports that the scombroid *Euthynnus affinis* may require as much as 10% increased lift after a meal, a need met primarily through increased swimming speed. *Euthynnus affinis* lacks a swimbladder; thus, increased swimming may be thought a somewhat specialized response to a change in density; but any physoclistous fishes would be expected to show some comparable response until swimbladder compensation can be achieved. Fry (1957) has shown that the metabolic cost of swimming varies approximately as the square of the sustained swimming speed. Thus, if sustained swimming speed increases with R, then α_R must increase more than proportionately. But in terms of equation 7, α_T increases in proportion to R. The consequence, from equation 8, is decreased K with increased R. Personal observation suggests that spontaneous activity in salmonids takes the form of intermittent, rather than sustained, swimming. Smit (1965) has shown the remarkably high metabolic expenditure occasioned by spontaneous, erratic swimming of goldfish, doubtless reflecting the additional cost of frequent accelerations. If salmon are comparable in this respect, the preceding mechanism is reinforced.

Regardless of the specific mechanisms involved, however, Kausch (1968) has shown that carp are appreciably more active when fed than starved, and that the respiration required to support activity increases more than proportionally to the level of activity. Thus both direct and indirect evidence indicates that increased R induces a more than proportional increase of α_R. I conclude that increased costs of spontaneous external activities can account for LeBrasseur's data specifically, and for the negative phase of the laboratory K-line in general.

Reference to the "negative phase" of a K-line is necessary. K must, of course, increase before it can decrease, because K is by definition zero at the maintenance ration. From equation 8 it can be seen that regardless of the relation between R and α_R, K must increase initially if K is ever to be positive. Either LeBrasseur's feeding levels were all at least moderately high, or the point of inflection to negative slope occurs close to the maintenance ration. The latter view is favoured by the work of Brown (1946), who observed high growth efficiencies at feeding levels close to the maintenance ration. The second recent experiment bears on this problem.

Brett et al.'s Sockeye Salmon

Explication of the work of Brett et al. (1969) is more difficult, as their published data are insufficient for reanalysis. They assigned rations in fixed proportion to body weight; thus, as before, we must assume that rations, body weight, and metabolic level were continuous variables, and that their effects will be confounded in the observations. It is clear, however, that the assigned rations were substantially less than in LeBrasseur's experiment. Although differences in experimental design make precise comparisons difficult, the maximum rate of growth observed by Brett et al. is approximately half that observed in the chum salmon. The difference appears attributable in part to the way in which rations were administered. "Excess" rations for the sockeye consisted in being fed to satiation three times daily, rather than being afforded constant access to rations. Paloheimo and Dickie (1965, 1966a, b) have demonstrated that this practice leads to an intermediate feeding level; therefore, even the highest feeding level in the experiment was presumably below the maximum. Moreover, the absolute amounts of ration provided, regardless of the different diet types, were considerably less than in LeBrasseur's experiment. Thus, although metabolic level must have increased with growth, it appears that the sockeye data were obtained at generally lower levels of metabolism. A second difference between the experimental procedures is also significant. The effects of spontaneous activity were reduced by subjecting the sockeye to a continuous water current, indicating that α_R was probably a less important factor. Sustained swimming would also have the overall effect of reducing the fraction of ration energy available to support growth.

In part, the sockeye data were said to be analyzed "in the manner of Paloheimo and Dickie." The description does not appear quite accurate, as the "constant" ration levels appear to have been derived by averaging a constantly increasing variable. Nevertheless, if we assume that rations and metabolism varied less within than among experimental groups, as seems likely, the K-lines provided may be approximately correct. These show an ascending limb, followed in most instances by an inflection to negative slope. As these curves reflect data acquired at low to intermediate ration levels, I interpret the ascending limbs as the positive phase required by equation 8. Equilibration of spontaneous activity has perhaps extended the

period of positive slope beyond what might have obtained without enforced swimming, although this view is of course conjectural. It is apparent, however, that the negative phase would have continued had rations been provided ad libitum.

Generalization to natural systems upon the basis of experiments such as this is risky when the observations reflect the combined effects of several variables. For example, the effect of temperature upon growth efficiency noted by Brett et al. could as easily be assigned to the fact that temperature is correlated with rate of growth, hence fish size, which in turn is confounded with metabolic level and growth efficiency because of the weight–proportional feeding regime.

Taken together, the two salmon studies describe the overall relation between growth efficiency and rations predicted by equation 8. Although differences in method caused different aspects to be emphasized in each experiment, both the positive and negative phases of the K-line have been demonstrated. Rather than being contradictory, the two experiments appear complementary and capable of apprehension in terms of the simple mechanisms described in equations 7 and 8. The general form of the K-line predicted by equation 8 finds additional support in Rafail's (1968) reanalysis of the Dawes (1930a, b) plaice experiments. Rafail projected K-lines with both positive and negative phases, similar to those described here. Dawes' experiments provided essentially two feeding levels only, maintenance and maximum; thus the sigmoid relation between growth increment and rations necessary to Rafail's conclusions is derived from fish adapted to only the two extreme ration levels.

Little has been said of the effects of the remaining parameters of equation 8, which indicates that prey size, g, can affect growth efficiency, as can Z, the cost per unit of prey capture. The parameter Z is difficult to delimit, but with the assumption that foraging costs arise largely from the accelerations needed to capture a food particle, Z will vary in proportion to WV^2, where V is the peak swimming speed. The high cost of erratic swimming reported by Smit was noted previously. If V is proportional to body length, which in turn is proportional to $W^{1/3}$, then Z will vary as $W^{5/3}$. Although the absolute cost of each food capture must be small, even if the additional energy costs required to overcome drag forces are included, it is clear that a diet composed of very numerous food particles will in aggregate become costly, particularly for larger fish. The parameter g, which is only effective if Z is significant, can take a wide range of biologically realistic values, perhaps most often between 1 mg and 10% of body weight. According to my equation system, the effect of foraging cost in an aquarium would be most evident in experiments providing a relatively small diet item to fish of a wide range of sizes.

Three prey types were utilized in LeBrasseur's experiment in an effort to reveal any potential effect of prey size upon growth efficiency. The original analysis by LeBrasseur showed consistent but small differences in K with different ration types, a trend persisting in the present reanalysis and confirming the view that the exigencies of foraging within the confines of an aquarium will not often be very great. Accordingly, although particle size and cost of capture are undoubtedly important in nature, careful design would be necessary to reveal their effects unequivocally in the laboratory.

Discussion

My primary aim in the preceding evaluation has been to illustrate the probable mechanisms underlying laboratory investigation of the relation between food and growth. I have attempted to show that except for an initial positive phase at low rations, the K-line as originally described by Paloheimo and Dickie is probably the general form to be encountered when appropriately sought. I further infer that the mechanism responsible for the laboratory K-line derives from the varying levels of spontaneous activity induced by different levels of ration.

It is necessary to distinguish clearly between growth efficiency measures determined in the laboratory and in the field. In natural systems, several parameters of apparently minor significance in the laboratory can be expected to exert major influence on growth efficiency. Among these are the parameters affecting foraging efficiency, such as prey mobility, abundance, and size. Alternatively, it also appears that a factor of major significance in the laboratory, the level of spontaneous activity, may be relatively unimportant to natural systems. It is therefore unreasonable to expect that correlations derived from laboratory studies typical of those made to date can have any necessary significance for natural systems.

The present paper reinforces the earlier conclusions of Paloheimo and Dickie, which pointed up the inutility of experiments that confound the effects of multiple variables. It is obvious that the simple correlational approach to laboratory investigation of food and growth should now be abandoned in favour of experiments designed to show the role of specific mechanisms.

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Prediction of Fish Growth Efficiency in Nature[1,2]

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Using an equation system based upon the requirements of searching for prey, and the physiological properties of the predator, the hypothesis that growth efficiency of pelagic predators may be importantly determined by the metabolic cost of searching for prey is examined. The equation system indicates that growth efficiency decreases when the prey organisms become either rare or small in relation to the size of the predator. The system also indicates that sustained growth requires increasingly large prey organisms even if these are increasingly rare. Review of empirical literature indicates that the system's predictions are in accord with observation.

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THE pattern of fish growth in nature reflects temporal variation in both the absolute quantity of ingested ration and in growth efficiency, the fraction of rations retained as body substance. Growth efficiency, K, is itself a function of ration level; thus, if the major determinants of K could be sufficiently understood for each period in the life history of a species, prediction of growth in specific environments would become a biologically meaningful undertaking.

Kerr (1971a) concluded that K is in part determined differently in nature than in the laboratory. Thus, the metabolic cost of "spontaneous" activity appears significant in most laboratory environments, but is likely to be largely supplanted by foraging activity in nature. In the present account, I build upon the rationale of the previous paper to provide a simple analytic model that enables prediction of K when constraints of searching for food are important, as in natural environments. I then evaluate the model's predictions in the light of empirical evidence.

The Model

The present model is essentially similar to that developed in my analysis of published laboratory studies (Kerr 1971a), except that I omit any provision for expenditure of metabolic energy on spontaneous activity, and attach considerable importance to the cost of searching for food organisms. To provide the simplest meaningful system, I postulate a pelagic predator that searches randomly in three dimensions for randomly distributed food organisms until satiated or otherwise limited by physiological capacity. For clarity, and to permit explicit numerical solutions, I have based the model upon a specific predator, the brook trout (*Salvelinus fontinalis*) by using physiological and other parameters provided in Kerr (1971a).

The total metabolism, T_T, routinely expended in nature consists for the most part of three elements: T_F, the cost of foraging activity; T_S, the standard metabolism; and T_C, the internal cost of food utilization; that is,

$$T_T = T_F + T_C + T_S. \qquad (1)$$

The foraging metabolism supports the cost of searching for food organisms, and is given by the time spent searching multiplied by the cost of swimming per unit time. The time spent searching is in turn equivalent to the total distance travelled divided by the rate of swimming.

The total distance travelled in foraging is easily computed as the number of food particles comprising a day's ration multiplied by Q, the mean distance travelled between them. Where R, the daily ration, and g, the mean size of food particle are both in grams, the number of food particles is R/g. Determination of Q depends on the distance, r, at which a predator is able to perceive a prey organism. If prey are sufficiently abundant that r is greater than the distance between neighbouring prey organisms, then search is unnecessary and Q is determined as the mean distance between nearest neighbours in the prey distribution. From Chandrasekhar's (1943) numerical solution of the Hertz distribution of the nearest neighbour in a random distribution of particles, we may write

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$$Q = 0.554 \left(\frac{g}{D}\right)^{1.5}, \quad (2)$$

where D indicates the density of prey in grams per cubic meter. If $Q > r$, however, I assume that a random search pattern obtains until a prey organism is within distance r of the predator. In this situation, the mean Q can be estimated by reasoning similar to that of Paloheimo and Dickie (1964). If the numerical density of prey organisms is represented by D/g, then the average volume containing just one food particle is given by g/D. The volume searched by a predator in travelling distance d is equal to $\pi r^2 d + \frac{4}{3}\pi r^3$. Setting this equal to g/D, we obtain

$$d = \frac{g}{\pi r^2 D} - \frac{4\pi r^3}{3\pi r^2}.$$

By adding an additional unit r to account for the distance required to contact a particle once detected, d becomes equivalent to Q and

$$Q = \frac{g}{\pi r^2 D} - \frac{r}{3}.$$

In the model, I have approximated Q when search is required as

$$Q = \frac{g}{\pi r^2 D}. \quad (3)$$

Certain assumptions are implicit in equation 3, for example treatment of the prey organisms as motionless points, but the attendant errors do not appear serious. In application to the model, I have used either equation 2 or 3 as appropriate to the conditions imposed.

Under some conditions it is enough in the model to merely limit r to some reasonable value that simulates restrictions imposed by the clarity of the environment. Usually I have imposed a maximum r of 4 m, a reasonable value for oligotrophic trout waters. Because equation 3 depends on r^2, I have supposed that identification is also a function of prey size and distance away from the predator; that is, I have required that r is limited as the tangent of some minimum angle subtended at the predator's eye by the prey. Although few available data describe visual acuity of fishes, I find that data provided by Beukema (1968) for the threespine stickleback (*Gasterosteus aculeatus*) feeding on three species of prey are described by

$$r = \frac{0.0124\ (g)^{1/3}}{\tan\theta}, \quad (4)$$

where $\tan\theta = 0.0096$, and the constant assumes spherical particles of weight g and neutral density. Nakamura (1968) observed that visual acuity of the skipjack tuna (*Euthynnus pelamis*) and the kawakawa tuna (*Euthynnus affinis*) increased with intensity of illumination from approximately 5–50 min of arc. Although neither illumination intensity nor target contrast are stated for the experiments I reanalyzed, the value obtained, 33 min of arc, is acceptably intermediate for use in the model.

Equations 2, 3, and 4 now permit estimation of Q under a variety of conditions, and the total distance travelled in foraging can be written as

$$\text{distance} = RQ/g. \quad (5)$$

I now derive an expression for rate of swimming. For most organisms body length is a power function of weight, where L is length, and W is body weight, a specific population of brook trout examined by Ricker (1932) is adequately described by

$$L = aW^b \quad (6)$$

in units of meters and grams, if a is 0.045 and b is 0.3322. Cruising speed of fishes is often found to be approximately proportional to body length. Thus from equation 6 we may write

$$\text{speed} = akW^b \quad (6a)$$

where k is an appropriate constant. The routine cruising speeds of fishes in nature are not well known. Certainly routine speed must be less than the frequently observed maximum sustained rate of several lengths per second. Data provided by Hergenrader and Hasler (1967) for yellow perch (*Perca flavescens*) in Lake Mendota suggest that 1 length per second may be a fair estimate. In most subsequent analyses I have adopted a standard swimming rate of 1 length per second, in which instance the appropriate value of k is 86,400 where speed is in units of meters per day. As will be discussed below, the value of k chosen does not affect the conclusions I shall draw from the analysis.

As Fry (1957) has shown for a number of species, the metabolic cost of sustained swimming at a given rate varies as the square of the speed, and can be expressed as

$$\text{swimming cost} = sW(\text{speed})^2 \quad (7)$$

when swimming cost is in units of kcal per fish/day. From unpublished data of J. M. Graham provided in fig. 25 of Fry (1957), I estimate that the value of s equals 9.0716×10^{-12} for the brook trout, when speed is in meters per day. There are less approximate and more unwieldy ways of estimating the cost of swimming, but equation 7 is adequate here.

Taking the distance foraged from equation 5, the speed from equation 6*a*, and the cost of swimming from equation 7, we may now describe the daily metabolic cost of foraging as

$$T_F = \frac{sQRW(akW^b)^2}{gakW^b}. \quad (8)$$

The remaining terms of equation 1 are provided in Kerr (1971a); thus we have

$$T_C = mR,$$

where the slope m is 0.2882 for the brook trout.

$$T_S = \alpha_S W^\gamma,$$

and

$$T_T = \alpha_T W^\gamma$$

where γ is 0.85, α_S is 0.015, and α_T can take values between α_S and 0.066.

Equation 1 can now be expanded to

$$\alpha_T W^\gamma = \frac{sQRW(akW^b)^2}{gakW^b} + mR + \alpha_S W^\gamma,$$

and solving for R yields

$$R = \frac{g(\alpha_T - \alpha_S)W^\gamma}{sQakW^{b+1} + mg}. \quad (9)$$

The balanced energy equation (Winberg 1956) can be written

$$pR = \alpha_T W^\gamma + \Delta W \quad (10)$$

where the constant p corrects for nonassimilation of rations and ΔW represents the growth increment. Division of equation 10 by R yields an expression of growth efficiency

$$K = \frac{\Delta W}{R} = p - \frac{\alpha_T W^\gamma}{R}. \quad (11)$$

Replacing the R of equation 11 with its formulation from equation 9 yields

$$K = p - \frac{\alpha_T(sQakW^{b+1} + mg)}{g(\alpha_T - \alpha_S)}. \quad (12)$$

Equations 9 and 12 enable the effects of the search constraints of foraging to be evaluated, and suffice as a statement of the model. Numerical evaluation of the equation system requires appropriate algorithms. Thus selection of equations 2 or 3 for computation of Q depends upon the assigned values of g and D, and the resulting search conditions. Also, it is necessary to ensure that the time spent foraging does not exceed the time available. I have found, however, that a time limit is necessary only when unrealistically low swimming rates are imposed. Under biologically plausible conditions, the predator is limited by physiological capacity or strongly negative K well before absurdities can occur.

Results

The general properties of equations 9 and 12 can be summarized briefly for the total range of biologically meaningful parameters. From equation 9, the acquired ration is proportional to the metabolic level; it follows that K increases hyperbolically with the levels of ration and metabolism. In Fig. 1, I have shown representatives of the family of hyperbolae generated by equation 12 with biologically plausible parameters for a 1000-g trout. As either resource density or prey size decrease, the distribution of K values is symmetrically depressed. The zero intercepts for K indicate that under most forage conditions a metabolic expenditure of approximately 1.5–2.5 times the standard level is required to provide a maintenance ration.

In Fig. 2, I have shown the effect on the K-curve of reducing r, the maximum range of prey detection, from 4 m, as in Fig. 1, to 1 m. The curves of Fig. 2 were chosen to depict the relation for large prey items, because the limiting visibility has no significant effect with small prey items. Figure 2 indicates that decreased prey visibility can decrease K significantly only when the prey resource is composed of relatively large, rare organisms.

Taken together, the representative curves shown in Fig. 1 and 2 indicate that a 1000-g trout must have access to relatively high densities of organisms of plankton size (g = 0.001–0.01 g) merely to survive, whereas large prey organisms (1–25 g) can support relatively high levels of K at much lower densities.

Assigning a fixed level of α_T permits assessment of the effects of predator size. Figure 3 indicates that at a fixed metabolic level of about 2.5 times basal and with constant resource conditions, K decreases with increasing body weight. It is clear that trout must have access to increasingly larger food organisms (which may, however, be relatively rare) if trout are to achieve large body size. Figure 3 indicates that growth cannot exceed approximately 1000 g on a plankton diet even when available at the relatively high density of 0.05 g/m^3, and good growth efficiency can be sustained by large trout feeding on large prey (25 g) that are only 1/50th as abundant.

Although only a few representative curves have been shown in Fig. 1–3, these are sufficient to illustrate the general properties of the model.

Discussion

Several components of the foraging process in nature are not considered in the present formulation. To the extent that the missing components would affect either equations 9 or 12, the equation system is therefore in error. Two such components deserve explicit mention.

In compiling the system, I have assumed that the costs of food capture are small relative to the energy costs of search. As search constraints alleviate, the effects of the search component, Q and prey size, g, become insignificant and both R and K approach limiting values. As the limiting values are approached, the cost of food capture becomes relatively more important and thus equations 9 and 12 may become increasingly inaccurate. It seems unlikely that the error could become serious, because capture costs might not be great if prey

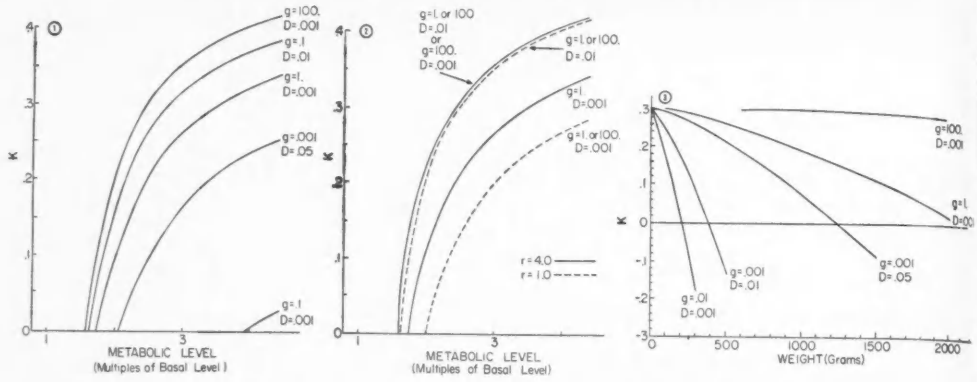

FIG. 1. Growth efficiency (K) as a function of metabolic level for a 1000-g trout, for different combinations of prey size (g, in grams) and prey density (D, in grams/m^3). Note that the distribution is symmetrically depressed as prey become small or rare.

FIG. 2. Relation of growth efficiency (K) to metabolic level for a 1000-g trout. Solid lines represent radius of prey detection (r) equals 4.0; broken lines, r equals 1.0. Note that the value of r becomes important as prey become large and rare (g is prey size in grams, D is prey density in grams/m^3).

FIG. 3. Relation of growth efficiency (K) to predator size for different combinations of prey size (g, in grams) and density (D, in grams/m^3). Metabolic rate is held constant throughout at about 2.5 times the basal level. If prey are individually large, low density levels suffice to maintain positive growth efficiency; at large predator size, small prey are required at much higher density levels.

were so abundant as to obviate search. Nevertheless, application of the equation system should be restricted to the more usual conditions, in which the predator population is sufficiently close to equilibrium with its prey that search costs are the prime determinant of growth efficiency.

Similarly, the model makes no provision for prey that are too large for a given size of predator. Schoener (1969) has provided a more general model of predation that specifies such functions. But the gain in generality achieved by Schoener is at the expense of the capacity to derive explicit numerical solutions. For present purposes I have omitted the relatively minor functions that cannot be exactly formulated for predatory fishes.

The aim of the modelling process is not to achieve complete isomorphism, but rather to provide sufficient approximation to reality that the salient mechanisms can be evaluated with insight. The criterion to be applied to the model is, therefore, whether the approximation achieved is sufficient. The equation system predicts that growth efficiency will decrease with decreasing prey density and size, and with increasing size of predator. If so, the postulated effects of each parameter should be ubiquitous in nature.

If prey size is important to growth efficiency, we would expect a general tendency among predators toward active selection of specific size classes of prey. Numerous studies of fish diets in nature indicate a common tendency for correlation of prey and predator size. Ricker (1932), for example, made specific note of the correlation between increasing size of brook trout and their prey, as have Leggett and Power (1969) and Smith and Page (1969) among others, for other species of predators. Such observations may indeed be archaic: the maxim "big bait for big fish" is a recurrent one in the popular angling tradition, and perhaps has been so for as long as men have fished. It remains to establish that size selection of prey is quantitatively verifiable.

Ivlev (1955) revealed marked electivity for prey size by several species of fishes in the laboratory, as have Beyerle and Williams (1968), Parsons and LeBrasseur (1968), and LeBrasseur (1969).

Two remarkable papers have demonstrated the importance of selection for prey size in nature. Brooks and Dodson (1965) showed that the alewife (*Alosa pseudoharengus*) and the blueback herring (*A. aestivalis*) were such effective size-selective predators that they controlled the species and size composition of the zooplankton in an array of New England lakes. Galbraith (1967) proved that yellow perch (*Perca flavescens*) and rainbow trout (*Salmo*

gairdneri) exhibited intensive selection for larger size classes of *Daphnia*, their major diet item.

From equation 12 the extreme instance is readily conceivable in which the food particle is so small that the assimilated caloric reward to the predator is insufficient to meet the cost of its capture. Although such a threshold has never been explicitly sought, the work of Galbraith suggests its existence at a biologically meaningful level. Galbraith observed that *Daphnia* were not utilized by either rainbow trout or yellow perch unless the individual prey exceeded a surprisingly clear threshold of 1.3 mm in length. Richman (1958) reported mean kcal values per gram dry weight of 4.1, 4.1, and 5.1 respectively for *Daphnia pulex* of mean length 0.7, 1.3, and 1.8 mm. I infer that the threshold for prey size described by Galbraith provides evidence that growth efficiency depends upon prey size, specifically because of the greater net ration-energy acquired with larger food particles.

There is adequate evidence, therefore, that predators tend to select large prey organisms in relation to their own body size. The effect of prey size upon growth efficiency is not simply dependent upon the relative sizes of prey and predator, however. The predator's radius of perception, r in equation 4, is approximately independent of body weight. For example, in the case of visual predation, visual acuity is primarily a function of pupil diameter, which changes relatively slowly with increasing body size. A large predator consequently searches less effectively than a small predator even when prey size is proportional to predator size.

The effect of prey size on predator efficiency seems, in part, comparable to the effect of prey schooling. Paloheimo and Dickie (1964), with the aid of a simple search model, examined the effect of schooling of prey upon the catch per unit time of solitary predators (fishing vessels). Their model indicates that increasing either the overall density of prey or the radius of the prey schools, augments, at a decreasing rate, the catch per unit time. (This effect can be seen from their fig. 2, but the reader is cautioned that the captions to their fig. 1 and 2 have been transposed.) Where the capacity of the predator is not limiting, increasing the radius of the prey school is comparable in effect to increasing the individual size of dispersed prey organisms. It would appear, therefore, that increases in prey density, prey size, and degree of aggregation may all increase the searching efficiency of the predator in similar ways. This generalization is in part supported empirically by the work of Ivlev (1955), who found that increased prey aggregation affected electivity in the same way as an overall increase in prey density. In this sense, the parameter D of the equation system can be regarded as accounting for prey aggregation, as well as density. Although schooling would therefore seem disadvantageous to the prey organism, when the prey aggregation bulks larger than the predator's capacity the schooling habit reduces the predator's efficiency (Brock and Riffenburgh 1959).

Although not specifically considered here, the effect of prey density upon the foraging success of predators is too well known to require documentation. Accordingly, I conclude that empirical evidence provides manifold support for the model's prediction that prey size and density and predator size will exert ubiquitous effects upon the growth efficiency of predatory fishes. The equation system is therefore at least qualitatively in accordance with observation.

The density effects predicted by my equation system may be profitably compared with the model of predation intensity developed by Ivlev (1955). In a series of laboratory experiments, Ivlev found that r, the ration consumed during a constant, relatively brief period of time was related to prey density, d, by an expression of the form

$$r = R(1 - e^{-xd}),$$

where R indicates the maximum possible ration and x is a fitted coefficient. Estimates of r as a function of d generated by my model are not adequately described by the Ivlev equation, for reasons that are readily apparent.

The Ivlev equation describes a time-limited system, and thus models the mechanical constraints of ingesting food particles from a bounded prey resource. My system models the physiological constraints of a predator searching in an unbounded random prey distribution. There is, therefore, no reason why the two models should be congruent.

The distinction is relevant, as some recent applications of the Ivlev model to vertebrates may not have satisfied its preconditions (Parsons and LeBrasseur 1968; Royama 1969). Clearly, choice of a predation model is contingent upon the relevant factors governing the specific predator–prey interaction. I suggest that the mechanisms inherent in the Ivlev model do not often control natural systems.

Direct tests of the numerical values of K predicted by the model require independent estimates of K and R for natural populations, but suitable data are lacking. Indirect numerical confirmation of the model can be made by comparing the growth trajectories of natural populations with computations based upon the model. The procedure is further elaborated on by Kerr (1971b).

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A Simulation Model of Lake Trout Growth[1,2]

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KERR, S. R. 1971. A simulation model of lake trout growth. J. Fish. Res. Bd. Canada 28: 815–819.

Kerr (J. Fish. Res. Bd. Canada 28: 809–814, 1971) described an equation system based upon metabolic constraints and foraging limitations that predicts level of rations and growth efficiency as a function of the availability of the prey resource. An algorithm that extends the equation system to a growth model is described here. With reference to increasing predator size, and to the mean size and abundance of prey, the model computes successive growth increments while holding the equations to the configuration that maximizes the ratio of growth efficiency to rations. When given information on the mean size and density of prey organisms, the model accurately predicts observed patterns of lake trout growth.

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FISHES characteristically exhibit an "open" or variable growth pattern in nature. Most currently available growth models are descriptive or correlational rather than analytic, and thus do not assist understanding of the biological processes underlying observed growth variability.

Kerr (1971) described an analytic system applicable to pelagic predators whose growth efficiency and ration level are primarily determined by success in searching for prey. The present paper shows that a growth model based upon the system is capable of simulating observed growth curves for naturally occurring lake trout. The major advantage of the growth model derives from its basis in biological mechanism, permitting a greater measure of insight into the organization of the production process.

Martin (1970) provides information that is particularly suited to evaluation of the model. His long-term study of the lake trout population of Lake Opeongo, Algonquin Park, Ont., indicates various changes, including increased rate of growth and age at maturity of the trout, subsequent to the introduction of a new forage species, the cisco *Coregonus artedii*. That is, a change in the composition of the prey resource modified the growth pattern of the trout. Martin's data are sufficiently detailed that the growth model can be used to simulate trout growth both before and after the cisco introduction, providing a unique test situation for the model.

Design of the Growth Model

The growth model incorporates the basic equation system described by Kerr (1971); however, I have altered numerical parameters as necessary to adapt the system to the lake trout (*Salvelinus namaycush* (Walbaum)). The numerical values used for the conversion, together with their literature sources, are set out in Table 1.

For a given size of predator, the basic equation system predicts the growth efficiency and ration level, using information on the size and abundance of prey organisms and the effect of environmental temperature on the metabolic parameters of the predator. The system can be applied to growth simulation by means of an algorithm that computes a growth increment for a given set of conditions, adds it to the body weight to produce a new body weight, and repeats the process for successive time intervals, changing environmental parameters as required, until a growth trajectory is synthesized for the desired time period.

The algorithm I have used also permits data tables provided by Martin to be used as input. The tables describe, for four length categories of lake trout, the relative frequency and average size of each of the prey species utilized by the trout. For use in the model, I have simplified the tables to include only the four forage species of major importance. From these data and the absolute density of prey, the frequency of predator contacts with prey and their average size can be computed for each of the four size classes of predator. Further, once the average spawning age has been reached, the algorithm deletes a fraction of annual production to account for loss of sex products. Again from Martin,

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TABLE 1. Listing of the parameters changed in converting the equation system of Kerr (1971) from brook trout to lake trout. For further explanation, units, and methods of derivation, the above reference should be consulted.

Symbol	Description	Value applied to		Literature source (lake trout)
		Brook trout	Lake trout	
s	Parameter of power function relating swimming speed to metabolic cost	9.0716×10^{-12}	1.1836×10^{-11}	Fry 1957
γ	Avg value for exponent of function relating metabolism and body weight	0.85	0.8	Winberg 1956
α_S	Intercept of power function relating standard metabolism to body weight	0.015	0.009	Estimated from data of Gibson and Fry 1954
α_A	Intercept of power function relating active metabolism to body weight	0.066	0.041	As above
a	Intercept of power function relating length to weight	0.045	0.0499	Composite value derived from Martin 1970
b	Exponent of function relating length to weight	0.3322	0.31603	As above

I estimated the reproduction losses to be approximately 10% of body weight annually for the sexes combined. The marked thermal preference of lake trout (Martin 1952) simplified the algorithm by allowing metabolic parameters to be set to 10 C for the duration of the growing season. The growing season in turn was determined as 6 months, to correspond to the icefree period. Although lake trout feed during the period of ice cover (Martin 1954), low environmental temperatures during that time slow metabolic processes, precluding significant amounts of growth. Indeed, the scale method of aging used for the Opeongo lake trout relies on a winter cessation of growth.

The standing crops of the several species of forage fishes utilized by the trout are unknown. Accordingly, I assessed the effects of a range of density levels. As will be discussed below, the system is relatively insensitive to prey densities in excess of low thresholds, thus the specific values assigned are not of immediate consequence.

The preceding elements of the model constitute straightforward utilization of information available on the biology of lake trout. The general structure of the algorithm is depicted schematically in Table 2. The scheme in Table 2 is specifically designed for the Opeongo data, although the routine can easily be adapted as appropriate to model other systems. For example, should environmental temperature vary sufficiently during the production season so that an average value cannot be used, a routine could be incorporated that selects metabolic parameters to correspond with ambient temperature. Indeed, any desired degree of detail can be incorporated, although it has been my experience that a relatively small number of factors suffices to account for observed growth patterns.

Level of Metabolism

The level of metabolism at which fishes routinely exist in nature is not known with certainty, but in the calculation system growth is importantly dependent on it. The level of metabolism affects

TABLE 2. General organization of the algorithm used for growth prediction. Details of the calculations are given by Kerr (1971).

1. Input all data:
   (*a*) the size classes of trout;
   (*b*) the frequency of occurrence of each prey species in each size class of trout;
   (*c*) the mean size of each prey species utilized by each size class of trout;
   (*d*) arbitrarily chosen density levels for the prey of each size class of trout;
   (*e*) metabolic and other parameters of the equation system.
2. For the appropriate size class of trout, refer to the input data and compute the mean size and availability of prey.
3. Determine the metabolic level that maximizes K/R.
4. Compute the daily ration and growth increment.
5. Compute the new body size of the trout.
6. Record the body weight and accumulated ration at regular time intervals.
7. If spawning age has been reached, delete a reproduction loss at the end of each year.
8. Return to 2, repeating the cycle until the desired time period is exhausted.

activity, hence ration intake, and in turn is affected by the ration level. Selection of metabolic level appropriate to the situations being modelled was guided by the following considerations.

If a prey resource is too rare, its predators cannot feed rapidly enough, on the average, to maintain their population. Conversely, the predator population will increase in response to increased prey abundance. In the words of MacArthur (1968) "the equilibrium level of the resource will normally coincide with the threshold level marking how few individuals the harvesters can use to perpetuate their population." That is, intraspecific competition tends to reduce the average ration available to a predator.

At the same time, natural selection will favour individuals that minimize the cost functional of ration acquisition. This application of the "Principle of Optimal Design" (Rashevsky 1960) indicates that selective advantage will accrue to the individual that maximizes its growth efficiency.

Combining these two considerations, we may expect that the individuals comprising a population that has established an approximate equilibrium with its resources will manifest two characteristics: a tendency to maximize growth efficiency, K, and a second tendency, imposed by intraspecific competition, to reduce rations, R, to the permissible minimum. The equilibrium solution to the two countervailing tendencies will be expressed as a tendency to maximize the ratio K/R.

In my equation system, both K and R vary with the level of metabolism, α (Kerr 1971). Accordingly, I assign α in the growth model by an iterative subroutine that selects the level of α and that produces the maximum value of K/R during each successive cycle of the algorithm.

The distributions of K/R for various types of prey resource are readily investigated by applying the iterative subroutine to the basic system given by Kerr (1971). The resulting estimates of routine metabolic level are of interest in themselves. When resource conditions are relatively favourable because of large prey size or high density, the maximum value of K/R is obtained at an α level corresponding to slightly more than twice the basal metabolic level (Fig. 1). As prey become rare, or very small relative to the predator, then the optimum level of metabolism becomes progressively higher.

Growth of Opeongo Lake Trout

The introduction of cisco to Lake Opeongo brought about two changes that affect the input to the growth model: the diet spectrum changed in species and size composition, and the age at sexual maturity increased. The changes are reflected in the corresponding growth curves predicted by the model (Fig. 2 and 3). In both figures, the solid lines represent the growth curves predicted for levels of prey density arbitrarily selected as "reasonable" but also chosen to provide acceptable fits to the individual points representing Martin's observations. On the basis of information on the prey resource, the growth model is therefore capable of describing the two different growth curves observed for the trout before and after the prey resource changed. To the first approximation the choice of density levels used is relatively unimportant. The broken lines (*a*) indicate the growth curves that result when improbably high prey densities are used (equal to 0.1 g/m^3). Clearly, the differences between the fitted and the excessive density levels are minor. In the case of the years prior to cisco availability, the fit is improved slightly by high prey density, except perhaps at the upper terminus of the curve, where the empirical data suggest an abrupt inflection. In the late years, the overall goodness of fit is slightly diminished by high prey densities. However, the small changes induced in either instance by relatively wide variation in ration density, from a fraction of a gram to 100 g/1000 m^3, suggest that prey density, once above some minimum threshold, may be relatively unimportant to trout growth.

The broken lines (*b*) and (*c*), which envelope the predicted curves, are both derived at the arbitrarily fitted prey densities, and represent respectively the upper limit of growth where the level of metabolism is maintained at a maximum, and a lower limit where metabolism is held throughout at about twice the basal level. Of the two limiting curves, the upper is perhaps more useful, because the asymptotes reached by both are undoubtedly artifacts of the coarse scale of the input data describing the diet. The length intervals used by Martin in his description of trout diet are separated by weight thresholds corresponding approximately to 500, 1500, and 3000 g body weight. It would have been preferable in the model to describe prey size and frequency of utilization as continuous functions of predator size, in which instance I expect the twice-basal metabolic level would have produced a closer correspondence to the observed growth. Regardless, the limiting curves indicate the wide range of growth trajectories potentially open to the model, and show that the good fits given to the empirical data are more than fortuitous.

The estimates provided by the model permit some further calculations of interest. Martin observed that fishery yield increased subsequent

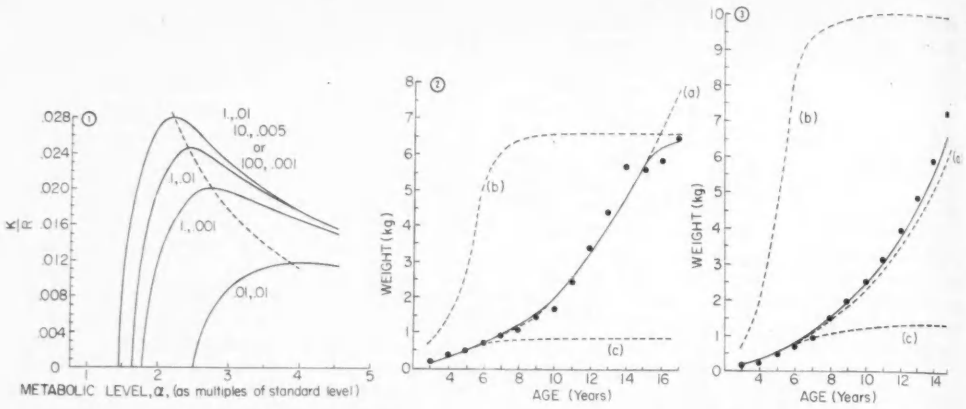

FIG. 1. Response to changes in metabolic level of the ratio growth efficiency/ration (K/R) for a 1000-g trout. Each curve was derived for a different resource type, where the first numerical value of the pairs shown by the curves is the mean particle size in grams, and the second is the prey density in grams/m³. Note that the maximum values of K/R, connected with a broken line in the figure, tend to occur closer to 2.1 times the standard metabolism as the prey resource becomes more available due to increased density or particle size.

FIG. 2. Growth curves for Opeongo trout prior to introduction of a new prey species. The solid line was generated by the model at prey densities of .0002, .00014, .0006, and .00035 g/m³, listed in increasing order for the four size categories of lake trout. The individual points represent values observed by Martin (1970). The broken line (*a*) is given by the model for any combination of density levels appreciably greater than those listed. The broken lines (*b*) and (*c*) represent limiting conditions, discussed in the text.

FIG. 3. Growth curves for Opeongo trout subsequent to introduction of a new prey species. The solid line was generated by the model at prey densities of .0001, .0002, .00035, and .0006 g/m³. See caption to Fig. 2.

to the cisco introduction. From his fig. 10, the increase may be roughly estimated as somewhat better than 30%, whereas the average weight of a trout appearing in the fishery increased from about 1.6 to 1.8 kg. The design of the growth model makes it possible to determine the cumulative ration required to grow a fish to any specified size, although it should be noted that the model as applied here considers only the icefree season, and thus underestimates the total ration. Nevertheless, comparisons will be approximately valid. The model indicates that the ration required to produce an average trout decreased from 10.4 to 9.4 kg in the same period. That is, the growth efficiencies computed on this basis increased from .154 to .192, an increase of about 25%. In view of the approximate nature of these calculations, the correspondence between the observed (30%) and predicted (25%) increase in trout production is close, suggesting that the enhanced trout production observed by Martin may be entirely accounted for by increased efficiency of the trout after cisco become available. Accordingly, it seems unnecessary to invoke alternative explanation for the increased trout production, such as changes in the basic productivity of the supporting food chain. Although approximate, these estimates are primarily intended to demonstrate that a growth model of this type makes it possible to analyze production systems in such specific terms.

Discussion

Given suitable information on the size distributions of the prey resource, the growth model has satisfactorily generated the observed growth patterns of Opeongo trout. The results indicate that a simple mechanistic model, incorporating a few functions describing foraging and physiological constraints, is capable of analyzing the growth process in nature with insight.

The model as applied here is unquestionably an oversimplified and approximate description of the complex system underlying growth in nature. Nevertheless, the satisfactory performance of the model suggests that the principal factors affecting growth may often be relatively simple, and thus amenable to analytic investigation.

Winberg (1956) has suggested that a metabolic level of about twice the basal rate is a useful average to apply to field conditions. Mann (1969) has reviewed subsequent evidence supporting Win-

berg's recommendation. The metabolic levels selected by the model varied continuously during the course of computing the growth curves, but nearly always remained within the range of two to three times the basal level. Thus, the estimates provided by the model correspond well with the available empirical evidence.

The performance of the model suggests that one may be justified in drawing other conclusions of interest to trophic ecology. It appears that prediction of piscivorous trout growth can be approximately independent of the density of prey, providing that a relatively low threshold abundance is met. Conversely, it appears that the size composition of the prey resource is a prime determinant of fish growth. If this is generally so, the variable form of observed growth curves acquires new meaning, and suggests that the production efficiency and size composition of exploited populations may be susceptible to management through control of the size composition of the supporting prey resource.

Kerr and Martin (1968) analyzed piscivorous and planktivorous populations of lake trout that provided similar fishery yields, although supported at different trophic levels. They concluded that the greater food resource available through the shorter food chain was used less efficiently, with the result that the potentially greater production of planktivorous trout could not be realized, and trout production therefore appeared independent of food chain length.

In the present analysis the converse situation appears. Introduction of cisco to the Opeongo trout did not affect the trophic level of the trout, thus their potential energy resource remained roughly the same. But the cisco modified the size composition of the prey resource, permitting the trout population a significant increase in growth efficiency. Consequently, trout production increased in Lake Opeongo, without any necessary change in the available energy resource.

The conclusions provided here require adequate evaluation in other production systems. Undoubtedly the precise form of growth model must vary as appropriate to the system and species under investigation. Nevertheless, if the analytic approach suggested here should prove to be generally applicable to fish populations, enhanced understanding of the production process and a greater measure of control seem attainable.

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Gillnet Selectivity on Sockeye (*Oncorhynchus nerka*) and Pink Salmon (*O. gorbuscha*) of the Skeena River System, British Columbia

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TODD, I. ST. P., AND P. A. LARKIN. 1971. Gillnet selectivity on sockeye (*Oncorhynchus nerka*) and pink salmon (*O. gorbuscha*) of the Skeena River system, British Columbia. J. Fish. Res. Bd. Canada 28: 821–842.

Exploitation of Skeena River sockeye salmon has been conducted almost solely by drift gillnets since 1875. This study was designed to determine the selective properties of nylon gillnets presently in use; to compare these with properties of linen nets used prior to 1955; and, using statistics of catch and escapement, to estimate the selective action of the fishery as a whole on sockeye and pink salmon in 1968.

Unique selectivity curves for nylon nets of $4\frac{5}{8}$–$5\frac{5}{8}$-inch mesh size could not be determined from sockeye catches. Mean size of age class 1.2 sockeye increased with mesh size but mean size of the predominant age class 1.3 sockeye demonstrated no trend. Age class 1.3 sockeye were among the largest on record and were too large to gill properly in all mesh sizes used.

Comparison of $5\frac{1}{4}$-inch mesh nylon gillnet with linen nets of $5\frac{5}{8}$-inch mesh used in the historic fishery was also influenced by the large size of age class 1.3 sockeye. Nylon nets were 2.5 and 2.7 times as efficient as linen for sockeye, and 8.0 and 9.0 times for pink salmon. Nylon gillnets caught larger sockeye and pink salmon than did linen. Variances of mean size were also greater for catches in nylon.

The statistics of catch and escapement for 1968 indicated that selection increased over the length range of age 1.2 sockeye but decreased over the length range of age class 1.3 sockeye. For pink salmon, which were extremely small in 1968, selection was increasingly large for larger size males and females. The length–girth relations of the species and sexes accounted for most of the difference between the selectivity curves. Retention by gillnets declined once the girth/mesh ratio exceeded 1.2 for sockeye. For pink salmon, no females were of a size to equal this ratio; the descending limb of the selectivity curve was due solely to males as the retention rates declined once girth/mesh ratio exceeded 1.0.

These findings suggest that in most years the gillnet fishery on the Skeena River would tend to select relatively larger sockeye salmon. In years such as 1968, however, selection would be against smaller fish. This frequent reversal combined with the intense modern fishery, which tends to remove virtually all fish during a short period and allows almost complete escapement in periods between fishing, suggests that selective fishing has probably not been a significant factor in decreased production.

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THE purpose of this study was threefold: (*a*) to determine the nature and degree of selection, with respect to sockeye and pink salmon, of nylon gillnets currently used in the Skeena River gillnet fishery; (*b*) to compare selective properties and relative efficiency of nylon gear with linen gillnets that were utilized prior to 1952; and (*c*) to describe the selective action of the fishery as a whole and to consider its possible long-term effects on the biological composition of the stocks.

Assessment of the selective action of the fishery has implications in assessing management practices. Shepard and Withler (1958) computed escapement levels prior to 1946 on the basis of estimates of annual rates of exploitation and the known levels of commercial catch. Age composition of the commercial catch was known, and proportions of each age class in the catch were applied to es-

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timates of escapement to establish a record of production for each brood year. Recent data indicate that age composition of the escapement, because of the selective nature of the gillnet fishery, differs markedly from that of the catch (Larkin and McDonald 1968). If the selective action of the fishery could be defined, brood escapements prior to 1946 could be adjusted to provide a more realistic picture of production levels that might change the nature of the stock-recruitment curve.

Selective Properties of Nylon Gillnets

Methods

Gillnets were chosen to represent, as nearly as possible, characteristics of the majority of nets used in the commercial fishery ($5\frac{1}{8}$-, $5\frac{1}{4}$-, and $5\frac{3}{8}$-inch stretched mesh). In addition, two smaller mesh sizes ($4\frac{5}{8}$ and $4\frac{7}{8}$") and one larger ($5\frac{5}{8}$") than the range found in the fishery were used, making a total of six mesh sizes. Measurements of 50 meshes from each size were taken periodically throughout the experiment to assess the validity of the manufacturer's stated size, and to determine whether changes in size occurred with use.

Nets were of the "river" type (constructed of finer material, lighter in color, and hung in to a greater extent than "outside" gillnets), hung in at a ratio of 3:1 (90 fath of stretched web to 30 fath fished length). All nets were fitted with swivel fasteners at both ends of cork and leadlines so that relative position in an array could be varied. Each set of lines was 32 fath long; thus, when the nets were assembled in array, a gap of 2 fath existed between each panel, reducing the possibility of fish leading along the graded series until they reached a mesh size suitable for gilling.

Experimental fishing was conducted during periods closed to commercial fishing. A total of six sets was made each day, each set employing a group of six nets of different mesh size. Fishing was conducted in a "river" area (Fig. 1), with the first set at about low tide and the last slightly before high tide.

The experimental fishing pattern was a modification of the Latin square design (Cochran and Cox 1957). Fishing was conducted on a total of 18 days. Each replicate comprised six sets on each of 6 fishing days. Each net panel was fished at each of six fishing positions relative to the boat each day; over the 6-day period each net was fished once at each possible combination of fishing positions and set locations. A total of three replicates was thus obtained in the 18 days fishing.

Although the Latin square design should not be systematized in any manner (Pope 1963), operation difficulties required that changes in the position of one net relative to the others be conducted in a systematic manner at the end of each set. There was thus the possibility of a systematic error if there were interaction

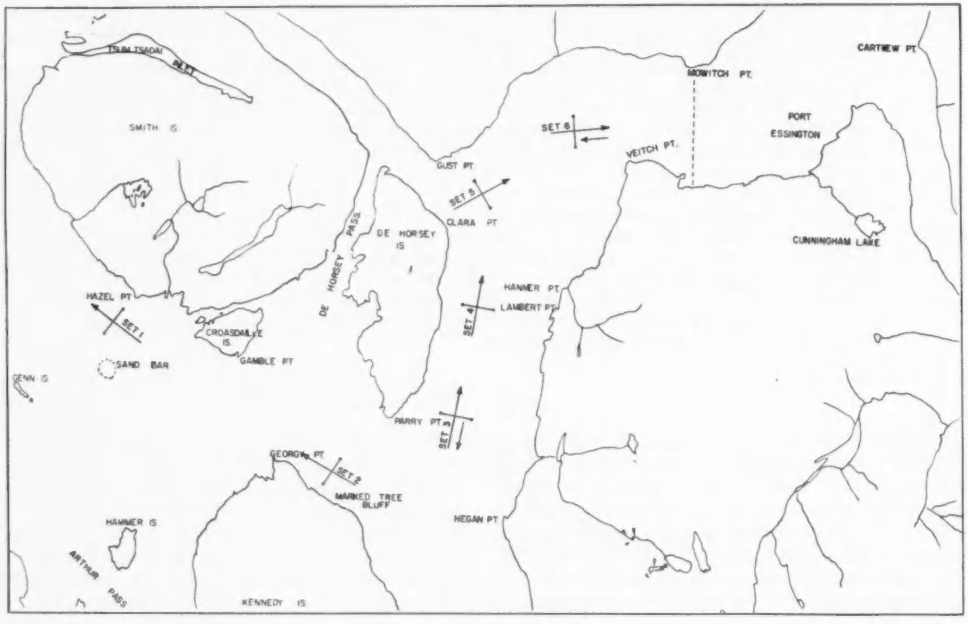

FIG. 1. Location of experimental fishing set areas for the nylon gillnet selectivity study at the mouth of the Skeena River.

between fishing position and set number, a possibility sufficiently remote to be ignored.

The initial position of the six nets relative to each other was established randomly prior to the start of experimental fishing, thus avoiding a regular progression of mesh sizes from one end of the series to the other. Although the relative position of one mesh size to another was not altered from the initial series, the initial fishing position for the series was changed each day; also, the initial fishing position for replicate II, day 1, set 1 was different from that of replicate I, day 1, set 1; and that for replicate III, day 1, set 1 was different from both.

Results

Mesh size measurements

Frequency distributions of deviations of mesh measurements from the manufacturer's stated sizes indicated little variation from stated sizes and showed no tendency to change as the season progressed. Average mesh sizes for the season (Table 1) were close to stated sizes. Conversions to perimeter measure (twice the stretched-mesh measurement) were based on stated sizes.

Numbers of fish caught

The total catch in the course of experimental fishing was 2302 sockeye and 356 pink salmon for which detailed length, age, and sex composition data are given in Tables 2–4.

For sockeye, the computed F ratios in analysis of variance are extremely small, and none is significant at $P \leq .01$; i.e., there were no significant effects of mesh size, fishing position, or set area on the catches of sockeye (Table 5). For pink salmon, only the set-area effect was significant.

It was expected that catches in each fishing position would reflect the setting procedure and thus the different lengths of time nets were fished in each position; i.e., the net in position 1 was the last in the water and fished less time than the net in position 2; and so on, with the net in position 6 spending the most time in the water. Although there were no significant differences, sockeye catches were largest near the center of the array, whereas for pinks the catch in position 1 was relatively low (Fig. 2).

The trend of the sockeye catches suggests that fish may have tended to "lead" from both ends of the array to be caught by nets in central positions. It is also possible that fish were not distributed randomly through the fishing area, and that the fishermen, being experienced, set the nets so as to put the center of the array in the best fishing area.

Because the test fishing was conducted in the closed time for commercial fishing, set area was not

TABLE 1. A comparison of the manufacturer's stated stretched mesh sizes with mean mesh sizes determined from sample measurements for six nylon gillnets.

	Mesh size					
Manufacturer's size (*inches*)	4⅝	4⅞	5⅛	5¼	5⅜	5⅝
Manufacturer's size (*mm*)	117.5	123.8	130.2	133.4	136.5	142.9
Mean size from samples (*mm*)	117.6	123.8	131.6	133.5	137.3	143.0

TABLE 2. Age and sex composition of sockeye caught in the nylon gillnet multimesh experiment (age designation after Koo 1962).

Mesh size (*inches*)	Age class												All age classes				
	Age 1.2			Age 1.3			Age 2.2			Age 2.3			Total	Total	Grand	Non-	Total
	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	total	aged	catch
4⅝	34	23	57	167	90	257	10	5	15	1	2	3	212	120	332	32	364
4⅞	41	35	76	172	87	259	6	2	8	1	3	4	220	127	347	47	394
5⅛	17	19	36	166	128	294	3	4	7	2	2	4	188	153	341	42	383
5¼	21	19	40	188	105	293	5	2	7	6	3	9	220	129	349	56	405
5⅜	11	20	31	166	107	273	4	2	6	5	3	8	186	132	318	29	347
5⅝	18	16	34	188	111	299	2	1	3	3	4	7	211	132	343	66	409
Totals	142	132	274	1047	628	1675	30	16	46	18	17	35	1237	793	2030	272	2302

TABLE 3. Mean postorbital–hypural length (mm) of sockeye caught in the nylon gillnet multimesh experiment by age and sex class.

Mesh size (*inches*)	Sockeye by age class[a]						Grand total[b]
	Age 1.2			Age 1.3			
	♀	♂	Total	♀	♂	Total	
4⅝	458.7	464.2	460.9	522.5	535.9	527.2	521.6
4⅞	465.1	451.7	458.9	520.0	535.7	525.3	508.6
5⅛	468.4	473.9	471.3	523.3	536.0	528.8	520.3
5¼	469.5	479.7	474.4	519.6	537.8	526.1	518.5
5⅜	476.5	476.5	476.7	524.1	540.2	530.4	524.1
5⅝	479.9	482.1	480.9	523.0	536.8	528.1	521.8

[a]Age classes 2.2 and 2.3 are not listed because of small sample sizes obtained in each category.

[b]Includes age classes 2.2, 2.3, and non-aged fish.

expected to demonstrate any marked effect on catch and was included in the experiment as a blocking variable. For sockeye there was no significant difference. For pinks, the significant result seems to have arisen from one particularly large catch in set area 1.

TABLE 4. Mean postorbital–hypural length (mm) and sex composition of pink salmon caught in the nylon gillnet multimesh experiment.

Mesh size (*inches*)	Numbers			Mean size		
	♀	♂	Total	♀	♂	Total
4⅝	110	118	228	395.2	400.7	398.0
4⅞	101	133	234	398.9	402.2	400.8
5⅛	45	121	166	404.5	411.8	409.8
5¼	27	99	126	406.0	412.3	410.9
5⅜	44	101	145	404.0	423.0	417.2
5⅝	29	82	111	412.9	426.1	422.6

It was anticipated that selective properties of individual mesh sizes would be sufficiently different to influence total catch in each mesh size. For sockeye (Table 2) there were virtually no differences in catch between the smallest net ($4\frac{5}{8}$″) and the largest ($5\frac{5}{8}$″). Pink catches (Table 4) were strongly suggestive of a decline in efficiency as mesh size increased, which was presumably obscured in the analysis of variance by the extreme variation in catches in individual sets.

Size, sex, and age composition

Spearman rank correlation coefficients (r_s) between mesh size as one variable and catches or mean length as the other variable, for each of sockeye and pink salmon, are listed in Table 6.

TABLE 5. Summary of results of analysis of variance for effects of mesh size, fishing position, and set number on numbers of sockeye and pink salmon caught in the nylon gillnet multimesh experiment (** denotes significance at $P \leq .01$).

Source	Sockeye				Pinks			
	Sum sq.	df	Mean sq.	F ratio	Sum sq.	df	Mean sq.	F ratio
Main effects								
A (mesh size)	13.87	5	2.775	0.16	121.90	5	24.380	0.60
B (fishing position)	71.11	5	14.220	0.85	17.51	5	3.502	0.08
C (set area)	28.21	5	5.642	0.34	2050.00	5	410.000	10.23**
Interactions (1st order)								
AB	363.00	25	14.520	0.87	1307.00	25	52.300	1.30
AC	82.81	25	3.312	0.20	145.50	25	5.823	0.14
BC	358.60	25	14.340	0.86	184.20	25	7.369	0.18
Interactions (2nd order)								
ABC	1491.00	125	11.930	0.72	4317.00	125	34.540	0.86
Error	7154.00	432	16.560		17300.00	432	40.040	

For sockeye of age 1.2 there is a significant negative relation between mesh size and numbers, in contrast to a positive but nonsignificant relation for 1.3 fish. The weakness of these correlations supports the conclusions of the analysis of variance in the previous section. For pink salmon the largest meshes tended to make smaller catches.

For both pinks and 1.2 sockeye of both sexes mean length increased with mesh size, but correlations for age 1.3 sockeye were nonsignificant. The overall value of r_s for all sockeye (.83), although barely significant, serves to demonstrate the strength of the trend for age class 1.2 in spite of the relatively small numbers involved (274 out of 2302).

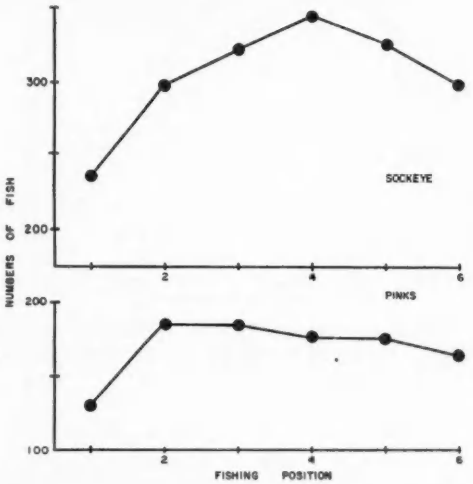

FIG. 2. Numbers of sockeye and pink salmon caught by fishing position in the nylon gillnet experiment.

TABLE 6. Spearman rank correlation coefficients between mesh size and mean length for sockeye and pink salmon caught in the nylon gillnet multimesh experiment (* denotes significance at $P \leq .05$).

Test	Sex class	Sockeye: Age class 1.2	Sockeye: Age class 1.3	Sockeye: Total	Pinks
Numbers	♀	−.66	+.29	−	−.83*
	♂	−.76	+.60	−	−.77
	Totals	−.83*	+.77	−	−.89*
Mean length	♀	+1.00*	+.37	−	+.83*
	♂	+.89*	+.77	−	+1.00*
	Totals	+.94*	+.49	+.83*	+1.00*

Gillnet selectivity curves

The normal probability model proposed by Holt (1963) and described by Regier and Robson (1966) provided the basis for computations of selectivity curves. Output from the computer program written for the technique included: calculated values of $R_{i+1,i,j}$ (ratio of numbers of fish of size l_j caught in meshes of size $i + 1$ and i) and their natural logarithms; $\hat{a}$ and $\hat{b}$ values (intercepts and slopes of regressions of $\ln R_{i+1,i,j}$ against l_j); a listing of the values estimated for the parameters $\hat{k}$ and $\hat{\sigma}^2$ and $\bar{l}_i$ of the selectivity curve equation for each set of paired comparisons; and calculated $\hat{S}_{ij}$ values (selectivity or relative efficiency of net of mesh size i for fish of size l_j) of paired comparisons.

Estimates of parameters of the selectivity curves for sockeye and pink length-frequency data in the simultaneous fishing experiments are given in Table 7.

Mean selection lengths for each net ($\hat{\bar{l}}_i$) for the sockeye do not increase progressively from the smallest mesh to the largest. The two estimates of mean selection length, for all nets except the smallest and largest, show wide discrepancies. For example, for 4¾-inch mesh, the value of $\hat{\bar{l}}_i$ (mean selection length) obtained from the comparison with 4⅜″ is 603.3 mm, whereas that from the comparison with 5⅛″ is 504.4 mm. Theoretically, the two values should be identical. These discrepancies, at least in part, are related to the various values of $\hat{b}$. They were significant and positive in two of the five comparisons. In the three remaining comparisons, the values of $\hat{b}$ were not significant, but negative, i.e., the larger mesh size caught smaller fish.

Changing minimum sample size from 5 to 10 in any given category for calculating ratios of catches did not alter these relations, nor did regrouping of the data into 2-cm length groups with a minimum sample size of 10. These changes altered calculated values of $\hat{\bar{l}}_i$ and $\hat{\bar{l}}_{i+1}$ in each instance, and consequently the selectivity curves for each mesh size for which estimates could be calculated (Fig. 3).

Changes in minimum sample size within categories from 5 to 10 radically altered estimates of curves for 4¾- and 5¼-inch mesh but did not change the general shape and location of the curves for 5¼″ and 5⅜″. It is possible that estimates for the latter two are fairly close approximations of their selective properties, since all three curves have roughly the same shape, and estimates of $\hat{\bar{l}}_i$ are within 1 cm of each other. Maximum efficiency for 5¼- and

TABLE 7. Estimated values of selectivity curve parameters based on sockeye and pink salmon length-frequency data. Analysis was on the basis of 1-cm length groupings with a minimum of five fish in each category for calculation of each $F_{i+1,i,j}$ (* denotes significance at $P \leq .05$).

Mesh size (*inches*)	Comparison	Sockeye						Pinks					
		$\hat{a}$	$\hat{b}$	$\hat{k}$	$\hat{\sigma}^2$	$\hat{\bar{l}}_i$	$\hat{\bar{l}}_{i+1}$	$\hat{a}$	$\hat{b}$	k	$\hat{\sigma}^2$	$\hat{\bar{l}}_i$	$\hat{\bar{l}}_{i+1}$
$4\frac{3}{8}$	$4\frac{7}{8}$					571.7	603.3					381.3	402.4
		0.594	−.001010	2.432	31290.0			−2.566	.006549*	1.622	3220.0		
$4\frac{7}{8}$	$4\frac{5}{8}$					603.3						402.4	
$4\frac{7}{8}$	$5\frac{1}{8}$					504.4	528.8					414.4	434.4
		−4.641	.008983*	2.034	2717.0			−8.232	.01939*	1.671	1033.0		
$5\frac{1}{8}$	$4\frac{7}{8}$					528.8						434.4	
$5\frac{1}{8}$	$5\frac{1}{4}$					604.0	620.3					557.1	572.1
		0.7746	−.001265	2.323	12850.0			−8.815	.001561	2.142	9608.0		
$5\frac{1}{4}$	$5\frac{1}{8}$					620.3						572.1	
$5\frac{1}{4}$	$5\frac{3}{8}$					537.1	549.2					395.9	404.8
		−4.442	.008179*	2.011	1475.0			−3.210	.00819	1.482	1109.0		
$5\frac{3}{8}$	$5\frac{1}{4}$					549.2						404.8	
$5\frac{3}{8}$	$5\frac{5}{8}$					575.2	602.6					429.6	450.1
		1.824	−.003097	2.107	8842.0			−5.763	.01310	1.573	1561.0		
$5\frac{5}{8}$	$5\frac{3}{8}$					602.6						450.1	

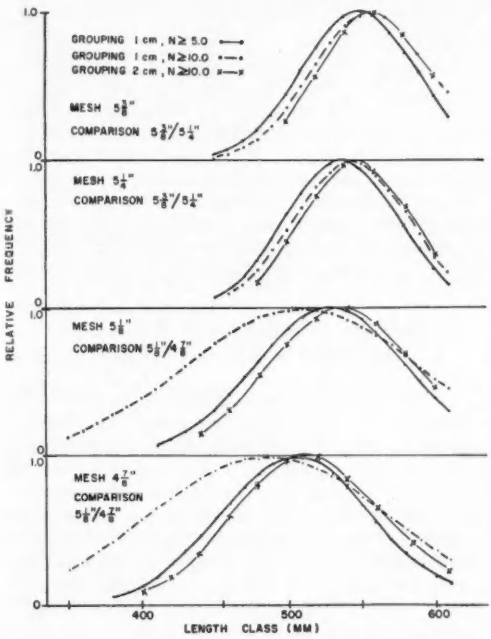

FIG. 3. Selectivity curves for experimental nylon gillnets based on length frequencies of sockeye catches.

5⅜-inch mesh occurs on sockeye of approximately 540 and 555 mm length, respectively.

Parameters and selectivity curves for pink salmon are given in Table 7 and Fig. 4. In each instance the values of $\hat{b}$ were positive and in two comparisons were significant.

Estimates of $\hat{\bar{l}}_i$ for pinks for each mesh size differ substantially from estimates for the same mesh size based on sockeye catches. For instance, $\hat{\bar{l}}_i$ for 4⅞-inch mesh based upon the 5⅛″/4⅞″ ratio was 504.4 mm for sockeye and 414.4 mm for pinks; similarly, values for 5⅛″ were 528.8 mm and 434.4 mm respectively.

Selectivity curves and length–girth relations

Characteristics of the selectivity curves for sockeye and pinks should differ only insofar as length–girth relations differ. Sockeye and pink length–girth relations as determined from samples collected in the commercial fishery are presented in a later section. Using the appropriate formulae for conversion, the lengths of pink salmon of equivalent girth were calculated for $\hat{\bar{l}}_i$ of sockeye for 4⅞- and 5⅛-inch mesh (Table 8). Although sockeye are longer than pinks of the same girth, the difference is not sufficient to explain the much greater $\hat{\bar{l}}_i$ for sockeye in both meshes.

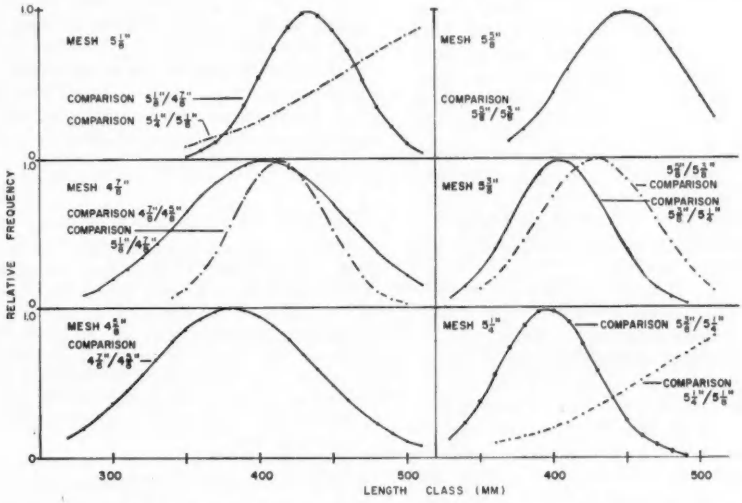

FIG. 4. Selectivity curves for experimental nylon gillnets based on length frequencies of pink salmon catches.

TABLE 8. Sockeye and pink salmon mean selection lengths ($\bar{l}_i$) and lengths of pink salmon of maximum girth equivalent to that of sockeye of mean selection length for 4⅞- and 5⅛-inch meshes.

Mesh size (*inches*)	Sockeye		Pink	
	$\hat{\bar{l}}_i$ (*mm*)	Girth (*mm*)	Length for equivalent max girth (*mm*)	$\hat{\bar{l}}_i$ (*mm*)
4⅞	504.4	327.2	487.7	408.4
5⅛	528.8	341.7	508.0	434.4

This magnitude of difference is suggestive of differential reaction of individuals of the two species to nets, or of a difference in swimming thrust between species, such as suggested by the studies of Garrod (1961), Konda (1966), and Lander (1969).

Summary comment on mesh size experiment

The mesh sizes chosen for this experiment ranged between 4⅞″ (11.75 cm) and 5⅝″ (14.29 cm), and in retrospect the differences between mesh sizes in the series may seem not to have been big enough for demonstration of selective properties of the various mesh sizes. However, results obtained by Peterson (1954) and discussed by Holt (1963) indicated that mesh differences of approximately 0.5 cm were sufficient to provide radical differences in the length-frequency distribution of sockeye catch. The nets used in Peterson's experiment were linen rather than nylon, and differences in elasticity may result in a broader range of selection within each nylon mesh size. Garrod's (1961) length-frequency data for *Tilapia esculenta* were radically different between mesh sizes of nylon gillnets that varied in mesh size as little as 0.5 cm. It thus seems likely that, in the present experiment, there should have been demonstrable differences in the length-frequency characteristics, at least in the catches in smallest and largest meshes.

Certain aspects of the results indicate that selective properties of the six mesh sizes differ. Fewer sockeye of age class 1.2 and pinks were caught with increase in mesh size; additionally, mean length for both groups increased with mesh size.

Catches of age class 1.3 sockeye were not significantly greater in larger meshed nets, nor was the mean length greater with greater mesh size. Because 1.3 sockeye were over three-quarters of the total sockeye catch, selective trends for the 1.2 fish were a relatively small feature in the total pattern. The size of 1.2 sockeye in 1968 was close to the average taken in the Skeena River gillnet fishery from 1915 to 1968. Sockeye of age class 1.3 in 1968 were larger than average — the fourth largest recorded for females and the fifth largest for males (Bilton et al. 1965, 1966, 1967, MS 1967, MS 1968). It is possible, then, that the 1.3 fish were, in effect, large in relation to all mesh sizes, and their capture was largely a matter of chance entanglement. In any event, from the experimental data it is not possible to construct a satisfactory set of selectivity curves with which it would be possible to improve age composition estimates of past sockeye escapements, such as those of Shepard and Withler (1958).

Relative Selectivity and Efficiency of Nylon and Linen Gillnets

Gillnet fishing has been conducted on the Skeena River since 1875 (Milne 1955), but few changes in fishing gear occurred prior to 1930. During the period 1930 to 1950, efficiency was increased by addition of engines and mechanical net drums to gillnet vessels, enabling fishermen to range more widely and make more sets (Shepard and Withler 1958). Concurrent improvements to gillnets consisted primarily of technical improvement of linen fibers. During the early 1950's synthetic fibers were introduced, and by 1955 virtually all linen nets had been replaced by nylon multifilament web. Since 1955, various other synthetics have been introduced, but in general, improvements have again been restricted to increase in tensile strength of fibers so that nets are now constructed of finer materials. Mesh sizes have differed between materials; linen nets were primarily 5⅛-inch (142.9 mm) stretched mesh, whereas the most common modern nylon net is 5¼-inch (133.4 mm) stretched mesh. Fortunately, linen nets constructed about 1945 were obtained in good condition and were made available for this study.

METHODS

A "river" and an "outside" type linen net (the two types differ in color; white in the river, green outside) were fished in an array with two modern nylon nets to test for differences in efficiency and selective properties between the two materials. The manufacturer's stated sizes were 5⅝″ for linen nets, 5¼″ for nylon "river" type nets. Measurements of 50 meshes from each of the four nets were taken periodically to assess validity of the manufacturer's stated size, and to assess changes in size with use.

All panels were hung to 50 fath of fishable length, to fish as in the commercial fishery; linen nets hung at a ratio of 2:1, nylon gillnets at the maximum allowable by regulation of 3:1. Each set of lines was 52 fath long, leaving a gap of 2 fath between panels.

The experimental design, a modification of the Latin Square (Cochran and Cox 1957), was followed in a manner similar to that for the nylon gillnet multimesh experiment. Each full fishing day required that a total of four sets be completed. Fishing was conducted for a total of 16 days. Each block, or replicate, comprised four sets on each of 4 fishing days. Thus, each net panel fished at each of four fishing positions relative to the boat each day; over a 4-day period, each net panel fished once at each possible combination of fishing positions and set locations. A total of four true replicates was thus obtained for the 16 days of experimental fishing. The relative position of the panels in the set was alternated to avoid bias. Fishing was conducted during periods closed to the commercial fishery at set locations 1–4 (Fig. 1).

Results

Mesh size measurements

Mesh measurements at four times during the course of the experiment (Table 9) differed significantly from the manufacturer's stated mesh sizes. Both nylon nets were slightly larger than stated, whereas both linen nets were slightly smaller. The nylon nets were not significantly different from each other, but the outside linen was smaller than the river linen. The difference was small and can be considered trivial.

TABLE 9. A comparison of the manufacturer's stated stretched mesh sizes with mean mesh sizes determined from sample measurements for two nylon gillnets and two linen gillnets.

	Nylon (1)	Nylon (2)	Outside linen	River linen
Stated mesh size				
inches	$5\frac{1}{4}$	$5\frac{1}{4}$	$5\frac{3}{8}$	$5\frac{3}{8}$
mm	133.4	133.4	142.9	142.9
Mean size from samples (*mm*)	135.0	135.4	139.1	139.6
SD	2.22	2.47	1.71	1.52
Confidence limits L_1	134.69	135.05	138.86	139.38
L_2	135.31	135.75	139.34	139.82

The difference between the nylon and linen nets is also sufficiently small that it would be a minor factor in selectivity and efficiency. Though the manufacturer's stated sizes indicate a difference of .375″ (approximately 9.5 mm), the real difference was only about 4.2 mm. Differences in catches between sets may thus be attributed primarily to the different materials.

Seasonal changes in either linen or nylon were not apparent. The results obtained for linen nets are at odds with those of Peterson (1954) where mesh sizes prior to use exceeded stated sizes; but shrinkage, mostly in the first week of use, reduced mesh sizes in most instances to about that stated. Measurements taken during this study were not initiated until the nets had been fished for 3 days, and thus a shrinkage may have occurred prior to initial measurements.

Relative efficiency of nylon and linen gillnets

For both sockeye and pinks the effect of net material on numbers caught was significant (Table 10) as was the first order interaction of net materials and set number for sockeye.

For the four panels the mean catches per set were:

	Nylon (1)	Nylon (2)	Outside linen	River linen
Sockeye	5.17	4.78	0.75	1.89
Pinks	1.53	1.34	0.11	0.17

TABLE 10. Summary of results of analysis of variance for effects of net material, fishing position, and set number on sockeye and pink salmon catches in the nylon versus linen gillnet experiment (* denotes significance at $P \leq .05$, ** at $P \leq .01$).

Source	Sockeye				Pinks			
	Sum sq.	df	Mean sq.	F ratio	Sum sq.	df	Mean sq.	F ratio
Main effects								
A (net material)	902.00	3	300.60	7.94**	108.80	3	36.29	5.72**
B (fishing position)	10.04	3	3.35	0.08	1.58	3	0.53	0.08
C (set number)	82.01	3	27.33	0.72	2.08	3	0.69	0.10
Interactions (1st order)								
AB	466.30	9	51.81	1.36	39.39	9	4.38	0.69
AC	657.60	9	73.07	1.93*	16.14	9	1.79	0.28
BC	152.70	9	16.97	0.44	31.57	9	3.51	0.55
Interactions (2nd order)								
ABC	614.90	27	22.77	0.60	115.40	27	4.28	.67
Error	7264.00	192	37.83		1217.00	192	6.34	

Mean catches of the two nylon nets (which were both of river type) were for sockeye 2.5 and 2.7 times as large as those for the river type linen, and for pinks, 8–9 times as large.

Differences between the two nylon panels were not significant for either species, nor were differences between the two linen panels, though the river linen caught more than twice as many sockeye as the outside linen.

Results for pinks suggest that nylon nets were in the order of 8–9 times as effective as linen nets. These figures are far greater than any previously reported for any species and may in part have been caused by the unusually small fish present.

Atton (1955) reported that for three mesh sizes nylon was more effective than cotton by respective factors of 2.6, 1.5, and 1.0 in catches of cisco and whitefish, and that the relative efficiencies approached unity as mesh sizes increased. The most effective nets he used, in terms of the size of fish available, were the smallest and the ratio of 2.6:1.0 compares closely with the above values of 2.5 and 2.7. Other assessments of relative effectiveness of nylon and cotton gillnets have provided similar results (Hewson 1951; Berst 1961; Pycha 1962).

The significant interaction of net type and set number was associated with maximum catches in set 1 for nylon nets, but set 3 for linen nets, and minimum catches in set 3 for nylon but set 1 for linen. The reasons for this difference are unclear, but clarity of water may have affected catches of the linen nets more than the nylon in sets 1 and 2 (see Atton 1955).

Relative selectivity of nylon and linen gillnets

The catches by the nylon and linen nets were similar with respect to the age and sex distribution of both pinks and sockeye (Table 11). All calculated values of chi-square were not significant.

Length-frequency distributions of sockeye and pinks caught in each of the four experimental panels (Fig. 5 and 6 respectively) also indicate similarity in the catches. Mean length of sockeye caught in nylon nets was slightly greater than for linen (Table 12), but within age and sex classes none of the differences were significant. In all instances but that of age 1.2 males for the nylon (1) versus river linen comparison, the mean length of sockeye caught in the nylon nets was slightly greater, in spite of the fact that linen nets were approximately 3% larger in mesh size than nylon. The same was true for pinks.

Because nylon nets have greater elasticity, consideration was also given to the possibility that the variance of sizes of fish they caught would be greater. However, in no instance were any of the F ratios significant.

Berst (1961) concluded that size composition of catches made with nylon and cotton nets did not differ significantly. Pycha (1962) concluded that

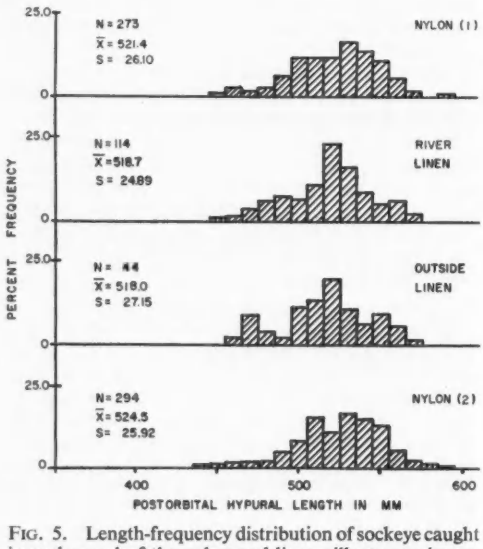

FIG. 5. Length-frequency distribution of sockeye caught in each panel of the nylon and linen gillnet experiment.

TABLE 11. Age and sex distribution of sockeye, and sex distribution of pinks in nylon and linen gillnet experiment.

	Pinks			Age 1.2 sockeye		Age 1.3 sockeye		All sockeye		
Panel	♀	♂	Total	♀	♂	♀	♂	♀	♂	Total
Nylon (1)	26	72	98	16	14	165	99	181	113	294
Nylon (2)	23	64	87	15	22	163	73	178	95	273
Outside linen	1	6	7	4	2	22	16	26	18	44
River linen	4	7	11	9	8	63	34	72	42	114

sizes of fish taken by equivalent mesh sizes of nylon and cotton did not differ greatly; his data, in fact, suggest that slightly larger fish were taken in the cotton nets, which is the reverse of the trend established here for sockeye and pinks. The differences established in this experiment might have been greater had there been no difference between the mesh sizes of the two materials used and, conversely, might have been less had the mesh sizes more closely agreed with the manufacturer's stated sizes. If the difference in mesh sizes is representative of the actual difference between material types used in the fishery, the conclusion may be reached that differences in selectivity are minor. Fishermen have apparently compensated for the increased elasticity of nylon nets to the point that the size characteristics of the catch and, consequently the age and sex characteristics, vary only slightly from what they were in the days of linen gillnets.

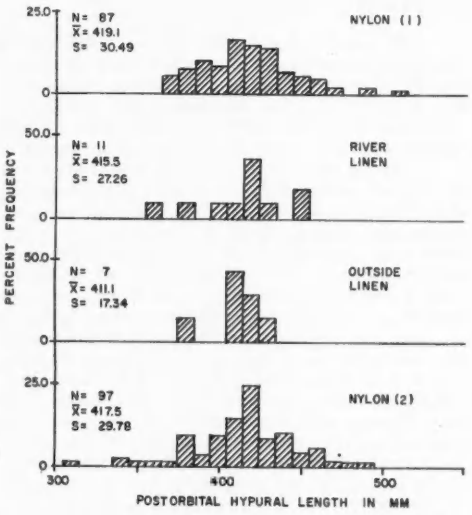

FIG. 6. Length-frequency distribution of pink salmon caught in each panel of the nylon and linen gillnet experiment.

Selective Action of the Commercial Fishery in 1968

The Skeena River gillnet fishery is coastal and in general concentrates near the river mouth; it may be considered to operate as a trap in that it is positioned across the major migratory routes. Effectiveness is such that continuous fishing during the migratory period could virtually eliminate the stocks. In consequence, fishing is permitted for a short time each week to ensure sufficient escapement to maintain spawning requirements. Some daily cohorts of inward migrating fish are thus virtually eliminated, whereas others are virtually untouched. This system of regulation has the effect of ameliorating selectivity by the gear, but may nevertheless involve some selection if there is a difference in the seasonal timing of various components of the fish stocks.

It is possible that the fishery exerts some form of sequential selection, i.e., if selection is for larger fish, the outer perimeter of the area should remove them differentially and the innermost segment of the fleet would catch smaller fish on the average. This process may not be detectable if the general availability of fish in the outer area fluctuates, or

TABLE 12. Sample size, average length, and variance of catches of sockeye and pinks by age and sex class for nylon and linen gillnets.

Species, age, sex	Nylon (1)			Nylon (2)			Outside linen			River linen		
	N	$\bar{x}$ length	S^2	N	$\bar{x}$ length	S^2	N	$\bar{x}$ length	S^2	N	$\bar{x}$ length	S^2
Sockeye												
Age 1.2 ♀	16	472.10	245.60	15	480.93	440.49	4	468.50	63.00	9	491.00	658.50
1.2 ♂	14	492.00	766.60	22	484.50	510.64	2	477.00	8.00	8	481.10	186.41
1.3 ♀	165	523.20	394.50	163	521.36	323.50	22	515.90	238.31	63	519.10	304.17
1.3 ♂	99	539.76	373.66	73	541.00	465.26	16	538.50	435.20	34	534.30	550.35
Total	294	524.48	672.25	273	521.42	681.37	44	518.02	737.53	114	518.72	619.78
Pinks												
♀	26	415.57	463.77	23	423.78	457.90		–			–	
♂	72	418.19	1034.58	64	417.46	1098.95		–			–	
Total	98	417.45	887.07	87	419.13	930.01	7	411.14	300.80	11	415.45	743.47

if a substantial proportion of the stock habitually travels through the outer fishing area at depths greater than gillnets will reach. In the innermost areas of the fishery, where gillnets for the most part fish the total depth of water, selection would primarily be a result of the inherent properties of the fishing gear.

Commercial exploitation of sockeye and pink salmon stocks bound for spawning areas in the Skeena River system takes place primarily in the drift gillnet fishery of British Columbia statistical area 4 (Fig. 7). To a lesser extent, Skeena stocks are intercepted by mixed purse-seine and gillnet fisheries located in statistical areas 3X and 3Y to the north; and in some years substantial numbers of pink salmon are taken by the purse-seine fishery in Ogden Channel and the gillnet fishery of Browning Entrance in statistical area 5 to the south (Fig. 7).

Commercial fishing within area 4 is restricted spatially by the seaward net fishing limits to the west and the upriver fishing boundary to the east; northern and southern limits are defined by boundaries with areas 3 and 5 respectively. The distribution of the fishing fleet has been characterized by a long-term trend seaward and the upriver fishing boundary has been moved seaward to compensate for increased efficiency of the fleet (Milne 1955; Shepard and Withler 1958). Since 1957 the fishing area has been stabilized and increased efficiency has been compensated by reductions in weekly fishing periods, and special closures when required.

Although nylon nets are used exclusively, their characteristics are not uniform throughout the fleet. Two distinct categories exist, characterized as follows:

(*a*) "Inside" or "river" nets: color — white to pale green; depth — 35–50 meshes deep; mesh size — predominantly 5¼"[1] (133 mm) stretched mesh; length — 550–600 fath of net per 200 fath fished length.

(*b*) "Outside" nets: color — dark green to black; depth — 60 meshes deep[2]; mesh size — predominantly 5¼" (133 mm) stretched mesh; length — 450–500 fath of net per 200 fath fished length.

"Inside" or "river" nets, as the name implies, are fished predominantly in the river and adjacent areas that are influenced by the silt load carried by the river (region I in Fig. 7). "Outside" nets are fished primarily in the open water beyond the major influence of silt-colored river water (region III). In region II, both gear types are used, primarily in nighttime fishing.

Other variations also occur within each major net type, such as minor differences in thread size, but by and large these variations were considered inconsequential by comparison with the major differences such as color and hanging ratios.

[1]Examination of sales records of two net companies indicates that 5¼-inch nets predominate in the Skeena fishery; a small number of 5⅛- and 5⅜-inch mesh sizes is also utilized.

[2]Maximum depth permitted by legislation is 60 meshes, regardless of mesh size.

Methods

In 1968, sockeye and pink salmon samples were obtained daily from each region from mixed boat catches. Postorbital to hypural plate lengths were measured to the nearest millimeter; sex was determined by examination of gonads; the "preferred" scale was taken for later determination of age; and maximum girths were measured (at the leading edge of the dorsal fin) from 100 sockeye per sampling period, with a girthometer like that described by Wydoski and Wolfert (1968). A further two girth measurements, in addition to maximum girth, were taken at the posterior edge of the premaxilla and at the insertion of head with body from a sample of Skeena River sockeye that had been preserved (frozen) for other purposes. These fish were thawed thoroughly before measurements were taken. Data were provided through the courtesy of H. D. Smith of the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

Sockeye escapement was sampled at the Babine Lake fence, through which, in recent years, 85–95% of the sockeye pass en route to spawning grounds. This escapement is representative of age classes 1.2 and 1.3 but not of the others that comprise a large proportion of the non-Babine stocks. Sampling at the Babine fence was at the rate of 0.5% on the count of the previous day. Sex of fish was determined from external characters, a procedure, which on checking, proved correct in 597 of 600 instances. Fork length was measured because postorbital–hypural plate measurements could not be taken easily from live fish. Both measurements were obtained from samples of fish of various age and sex classes and the linear relation between the two was used to convert fork length to postorbital–hypural lengths (Fig. 8).

To determine the freshwater ages of sockeye sampled at the Babine Fence, a preferred scale taken from the left side of the fish was examined. Saltwater ages were interpreted by examination of the second and third rays of the left pectoral fin of each fish (Bilton and Jenkinson 1969). To assess the reliability of the age estimates so derived, the readings were compared with those derived from otoliths taken from among the sampled fish at the rate of 100 per week. The comparison suggested that, if otolith readings were used as the criterion, scales and fin-ray readings provided reliable estimates of fresh- and saltwater ages. Consequently, readings from scales and fin rays were used to estimate the age composition of the escapement at the Babine fence.

Escapements of pink salmon in all major spawning areas were sampled for size and sex characteristics during

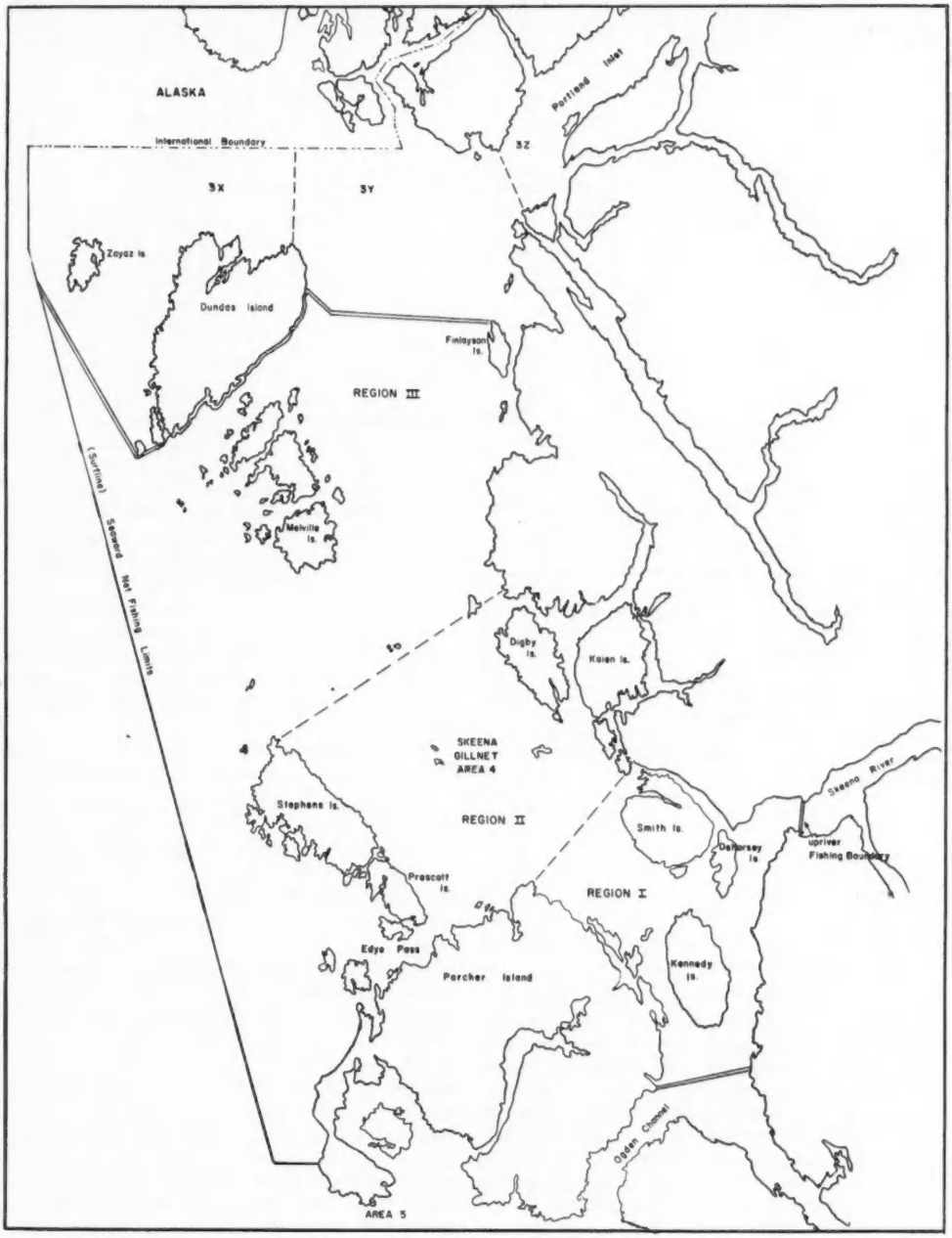

FIG. 7. The commercial fishing area adjacent to the Skeena River, and subareas where specific gear types operate.

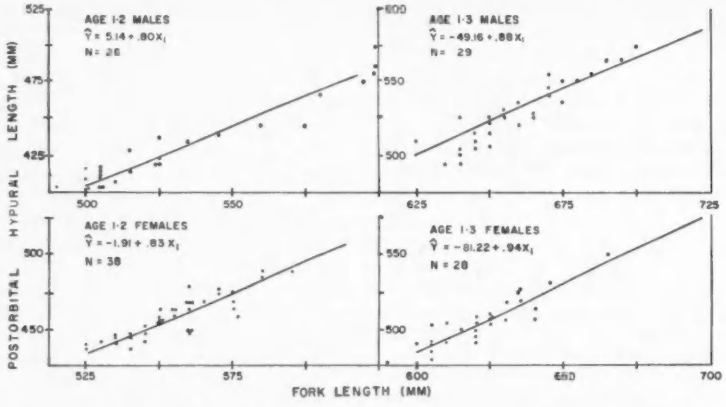

FIG. 8. Relations between fork length and postorbital–hypural plate lengths by age and sex class for Babine Lake sockeye.

the course of population enumeration. In 1968 the pink escapement to Lakelse River was 1,200,000 and the total to the Skeena region 1,700,000. Sampling was accordingly intensive on the Lakelse River. Periodic measurements of fish in other systems (Kitwanga and Kispiox rivers primarily) indicated that size was not substantially different from Lakelse samples and the latter were therefore assumed to be representative of the total escapement.

Age, size, and sex compositions of the 1968 pink and sockeye stocks were established by combining length-frequency distributions of catch and escapement. The selective action of the fishery was determined through use of the direct method of Regier and Robson (1966); i.e., where

$$\hat{s}_{ij} = \frac{n_{ij}}{\hat{N}_{ij}}; \text{ and } \hat{S}_{ij} = \frac{\hat{s}_{ij}}{\max \hat{s}_{ij}}.$$

RESULTS

Age, sex, and size characteristics of sockeye and pink salmon commercial catches

In the commercial catch of sockeye (Table 13), trends in size between areas of capture were not uniform for various age and sex classes. Both male and female 1.2 sockeye were largest for region III (the outer perimeter of the fishery), intermediate in region II, and least in region I (the river fishery), with differences between mean lengths in each instance amounting to approximately 5 mm. Sockeye of age 1.3 of both sexes were largest in region III, but smallest in region II. The differences were small, about 2 mm or less.

Age 2.2 fish were present in small numbers, were intermediate in mean size between 1.2 and 1.3 sockeye, and, perhaps as a result of small sample sizes, showed different trends for the sexes. The largest females were taken in region III and smallest in region II, but males were largest in region II and smallest in region I.

Sockeye of age class 2.3 were larger than those of age class 1.2 and 2.2 but not as large as those of age 1.3. In this group the largest females were in region II and the smallest in region I; for males, the largest were in region III and the smallest in region I.

In total aggregate, there was virtually no difference in the size of sockeye in the three regions.

Pink salmon (Table 14) also exhibited a slight decrease in mean size of capture from region III to region I when samples of both sexes were combined for each region. As for sockeye, differences were slight and generally less than 2 mm.

Commercial catch samples arrayed by week of capture and grouped for all regions of capture (Table 15), show little change in the week-to-week age composition. In general, age 1.2 fish represented about 11% of the weekly catch, age 1.3 about 82%, and others about 7%.

The pattern of exploitation in 1968 was fairly regular, in that at least 2 days fishing per week was permitted. Tagging experiments have shown that sockeye take an average of 4 days to travel through the fishing area so that fish entering the area on any given day would be available to some degree in at least one segment of the fishery. Differences in availability because of the intermittent nature of the fishery (i.e., within a week) would thus tend to mask the sequential pattern of selection.

TABLE 13. Age, sex, and size composition of the area 4 commercial sockeye catch by region of capture.

Region of capture	Age 1.2			Age 1.3			Age 2.2			Age 2.3			Totals		
	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total	♀	♂	Total
Inside (I)															
No.	140	168	308	1263	809	2072	36	37	73	34	31	65	1473	1045	2518
%	5.56	6.67	12.23	50.16	32.13	82.29	1.43	1.47	2.90	1.35	1.23	2.58	58.50	41.50	100.0
Mean length (*mm*)	466.3	465.3	465.8	518.3	536.5	525.4	491.2	483.3	487.2	507.6	525.5	516.1	512.4	522.8	516.8
Mixed (II)															
No.	124	145	269	1152	709	1861	40	28	68	19	13	32	1335	895	2230
%	5.56	6.50	12.06	51.66	31.79	83.45	1.79	1.26	3.05	.85	.59	1.44	59.87	40.13	99.99
Mean length (*mm*)	471.1	470.2	470.6	518.2	534.0	524.2	483.5	494.6	488.1	517.4	531.9	523.3	512.8	522.4	516.7
Outside (III)															
No.	154	167	321	1073	621	1694	52	60	112	35	30	65	1314	878	2192
%	7.03	7.62	14.65	48.95	28.33	77.28	2.37	2.74	5.11	1.60	1.36	2.96	59.95	40.05	100.0
Mean length (*mm*)	473.7	479.7	476.8	519.9	537.1	526.2	497.6	486.6	491.7	516.6	536.0	525.6	513.5	522.7	517.2
Total															
No.	418	480	898	3488	2139	5627	128	125	253	88	74	162	4122	2818	6940
%	6.0	6.9	12.90	50.3	30.8	81.1	1.8	1.8	3.6	1.2	1.1	2.3	59.4	40.6	100.0
Mean length (*mm*)	470.4	471.8	471.1	518.8	535.8	525.3	491.4	487.4	489.4	513.3	530.9	521.4	512.9	522.6	516.8

Selectivity of the fishery on sockeye and pink salmon

The age and sex composition of the Babine escapement in 1968 (Table 16) was in total 18.6% age class 1.2, 80.2% age class 1.3, and 1.2% others. The first 2 weeks of the run contained a higher proportion of 1.2 fish than the middle and later parts of the season, but in general the season had a relatively uniform pattern. The catch was 60% of the total stock.

Superimposed on the length-frequency distributions of the sockeye catch and escapement (Fig. 9) are estimates of the percentage length-frequency distribution of the total sockeye stock obtained by summing calculated catch and escapement within each length class. For fish 450 mm in length and less, and for fish greater than 540 mm, the proportion in the catch is less than the proportion in the total stock. The smallest and largest sockeye were caught somewhat less frequently than their abundance would indicate. For fish between 460 and 540 mm the reverse is true; a greater proportion occurred in the catch than in the total stock.

Estimates of the selectivity indices ($\hat{s}_{ij}$), to which a selectivity curve has been fitted by eye (Fig. 10), indicate that selection by the fishery in 1968 is suggestive of a bell-shaped curve with an extremely broad dome ($\hat{s}_{ij}$ ranges between .60 and .64 over the range of fish sizes of 500–540 mm). There is a suggestion of skewness in the same direction as that identified for whitefish data by Regier and Robson (1966), the right limb of the curve beyond 540 mm falling more steeply than the left limb rises.

The broadness of the dome of the curve is not typical of most instances reported, and probably reflects different mesh sizes and types of net construction. To some degree it may also reflect the efficiency of modern nylon gillnets, which for the river fishery in particular are hung in to a large degree and are constructed of fine twine.

The selectivity curve comprises four age–sex class components, each of which has a different length-frequency distribution. Values of s_{ij} for males and females of age class 1.2 (Fig. 11) increased almost linearly over the length distribution. For fish 430 mm and larger, the rate of selection on males was greater than for females. For age class 1.3 the rate of selection was the same for males and females, and decreased over the greater part of the length range (Fig. 12). The curve of Fig. 10 is thus a composite, the ascending limb indicating selection of 1.2 sockeye, the dome and descending limb the selection of 1.3 fish.

Pink salmon data presented in comparable fashion indicate a selection far stronger than for sockeye. Fish up to 380 mm represented a much greater proportion in the total stock than in the

TABLE 14. Sex and size composition of the area 4 pink salmon gillnet catch by region of capture.

Region of capture	♀			♂			Total		
	No.	%	$\bar{x}_L$	No.	%	$\bar{x}_L$	No.	%	$\bar{x}_L$
Inside (I)	140	28.0	406.3	360	72.0	412.1	500	100.0	410.5
Mixed (II)	139	29.8	407.0	360	70.2	413.5	513	100.0	411.1
Outside (III)	94	31.3	416.4	205	68.7	412.4	299	100.0	413.7
Totals	373	28.7	409.1	926	71.3	412.7	1299	100.0	411.7

TABLE 15. Age and sex composition of the 1968 weekly commercial sockeye catch.

Week	Age 1.2				Age 1.3				Age 2.2				Age 2.3			
	♀	♂	Total	%	♀	♂	Total	%	♀	♂	Total	%	♀	♂	Total	%
June 24–30	36	46	82	16.11	196	151	347	68.17	11	24	35	6.88	23	20	43	8.45
July 1–7	48	90	138	12.67	523	347	870	79.89	26	21	47	4.32	15	15	30	3.76
8–14	31	57	88	11.89	331	274	605	81.76	7	15	22	2.98	10	14	24	3.24
15–21	121	110	231	11.67	1013	642	1655	83.63	34	26	60	3.03	12	17	29	1.47
22–28	89	103	192	12.06	860	450	1310	82.33	34	28	62	3.90	20	5	25	1.57
July 29–Aug. 4	34	27	61	11.59	300	152	452	85.93	5	3	8	1.52	3	1	4	0.76
Aug. 5–11	59	47	106	20.16	266	123	389	73.95	11	8	19	3.61	5	3	8	1.52
Totals	418	480	898		3489	2139	5628		128	125	253		88	75	163	

TABLE 16. Weekly age and sex composition of the 1968 Babine Lake sockeye escapement.

Period	Age class 1.2 ♀	♂	Total	%	Age class 1.3 ♀	♂	Total	%	Age classes 2.2+2.3 ♀	♂	Total	%
July 15–21, 22–28	18	15	33	34.0	40	20	60	61.9	2	2	4	4.1
July 29–Aug. 4	16	23	39	39.4	35	23	58	58.6	0	2	2	2.0
Aug. 5–11	54	45	99	18.2	297	145	442	81.4	2	0	2	0.4
12–18	59	44	103	19.5	271	147	418	79.2	4	3	7	1.3
19–25	28	19	47	12.0	223	118	341	87.2	3	0	3	0.8
Aug. 26–Sept. 2, Sept. 3–14	29	17	46	14.8	184	75	259	83.3	4	2	6	1.9
Totals	204	163	367	18.6	1050	528	1578	80.2	15	9	24	1.2

catch, whereas fish longer than 380 mm were caught at a higher rate than abundance would indicate, had there been no selection (Fig. 13). The effect of this selection on size composition of the escapement was not marked because the catch was just 18% of the stock.

The selectivity curve is suggestive of a bell-shaped curve with a steep ascending limb (Fig. 14). The dome is narrower, with max $\hat{s}_{ij}$ (between .39 and .41) occurring over the 3-cm range between 420 and 440 mm, and surprisingly, the descending limb begins at less than 450 mm, at a much smaller size than for sockeye (about 500 mm, Fig. 10).

The shape of the selection curves is different for male and female pinks. Females smaller than 380 mm were virtually unexploited, but above that size were caught at an increasing rate throughout the remainder of the length range. Males between 350 and 400 mm were increasingly selected and at a higher level than for females, but beyond 400 mm, where selection continued to increase for females, it decreased for males.

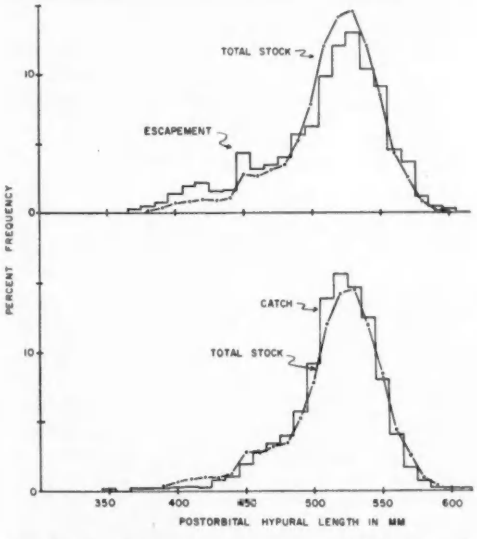

FIG. 9. Percentage length-frequency distribution of the 1968 sockeye catch, escapement, and total stock.

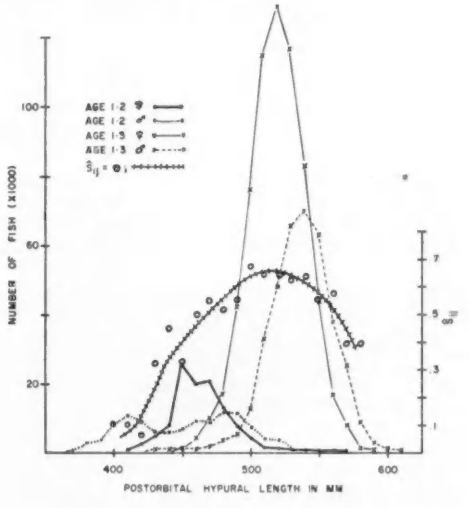

FIG. 10. Length-frequency distribution of sockeye by age and sex class, and the composite selection curve imposed by the fishery in 1968.

Selectivity and length–girth relations

Equations of the lines relating maximum girth to postorbital–hypural length for salmon taken during the field season are given in Table 17, and those for girths at the premaxilla, the insertion of head and body, and maximum girths for the frozen fish samples are given in Table 18.

Freezing and thawing altered the body shape of the sockeye. For example, maximum girth for an

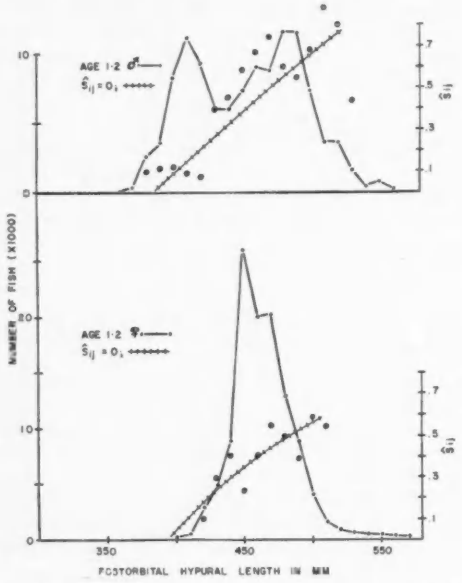

FIG. 11. Length-frequency distributions and selectivity indices for age 1.2 female and male sockeye.

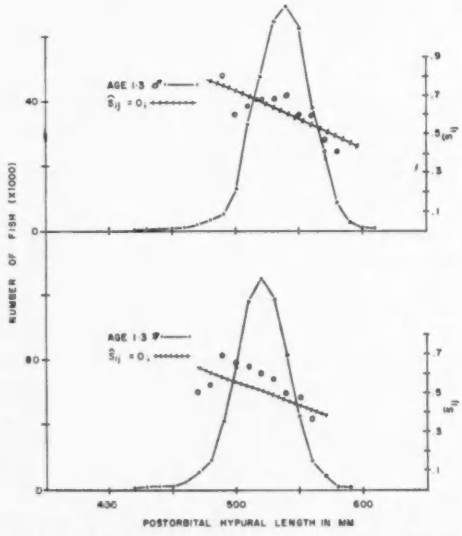

FIG. 12. Length-frequency distributions and selectivity indices for age 1.3 female and male sockeye.

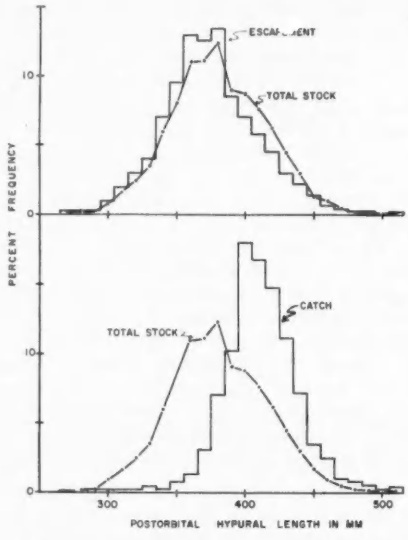

FIG. 13. Percentage length-frequency distribution of the 1968 pink salmon catch, escapement, and total stock.

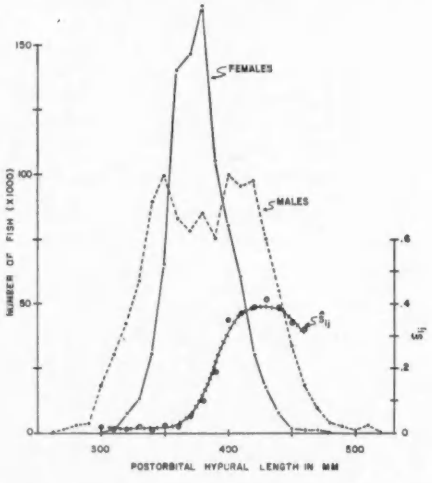

FIG. 14. Length-frequency distributions of pink salmon by sex class, and the composite selection curve imposed by the fishery in 1968.

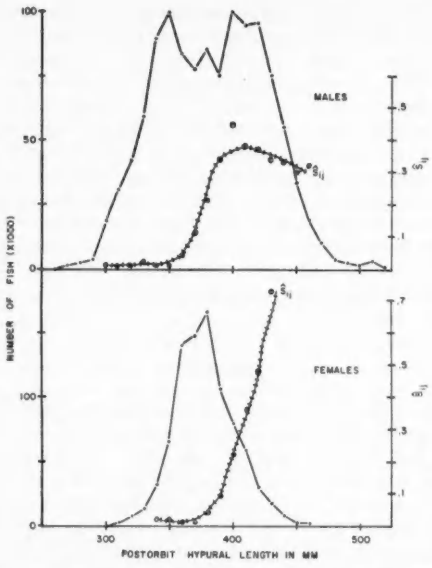

FIG. 15. Length-frequency distributions and selectivity indices for female and male pink salmon.

TABLE 17. Maximum girth (Y) to postorbital–hypural length (X) for sockeye and pink salmon sampled in the commercial fishery.

Species	Group	Straight line relation	N	Mean length (*mm*)
Sockeye	1.2 ♀	$y=-15.57+.66X$	47	466.7
	1.2 ♂	$y=-21.24+.68X$	70	467.4
	1.3 ♀	$y=50.97+.54X$	80	524.7
	1.3 ♂	$y=18.89+.62X$	80	535.8
Pinks	♀	$y=-11.10+.67X$	38	413.3
	♂	$y=-28.32+.75X$	62	416.6

age 1.2 female of length 400 mm would be 248 mm using the equation of Table 17, but 272 mm using the equation of Table 18 for frozen samples. However, fish that were frozen had been measured for postorbital–hypural length at the time they were obtained from the commercial catch, and it is apparent that shrinkage in length occurred as a result of freezing and accounts for the discrepancies. For the calculations that follow, unless otherwise noted, lengths for frozen samples were taken as the length before freezing.

McCombie and Berst (1969) demonstrated that efficiency of nylon nets to retain fish of three different species was optimum when the ratio of maximum girth to mesh perimeter ranged between 1.0 and 1.2. Beyond 1.2 for two species of the three, virtually no fish were retained, and for the third species no fish were retained beyond 1.6. If this sort of relation applied to sockeye and pink salmon, then differences in girths between species and between age and sex classes within species might explain the different selectivity curves for the various age–sex groups.

The most common gillnets utilized in the fishery had a mesh perimeter measure of 266 mm. From the maximum girth–length relations derived from fish sampled in the field, for fish having girths of 266 mm:

	Age & sex	Length of fish with maximum girth (*266 mm*)	Length for optimum girth to mesh perimeter ratio ($1.2\times266=319$)
Sockeye	1.2♀	426.6	506.9
	1.2♂	422.4	500.4
	1.3♀	398.2	496.4
	1.3♂	398.6	484.0
Pinks	♀	413.6	492.7
	♂	392.4	463.1

For all age classes of sockeye, the maximum values of $\hat{s}_{ij}$ (Fig. 11 and 12) are at lengths that agree closely with those expected for a girth:mesh

TABLE 18. Sockeye girth (Y) and postorbital–hypural length (X) relations from frozen specimens that had been thawed.

Age–sex group	Premaxilla	Head and body	Max	Mean length after freezing and thawing (*mm*)	Mean length before thawing (*mm*)	N
1.2 ♀	$y=14.73+.35X$	$y=8.24+.54X$	$y=72.16+.50X$	458.9	471.1	50
1.2 ♂	$y=-10.45+.43X$	$y=-35.02+.66X$	$y=-53.40+.78X$	451.1	459.1	44
1.3 ♀	$y=-2.99+.40X$	$y=30.20+.51X$	$y=-23.59+.71X$	507.6	516.3	50
1.3 ♂	$y=-28.18+.48X$	$y=-42.62+.68X$	$y=-51.80+.78X$	521.4	533.4	50

perimeter ratio of 1.2:1. The selection exerted by the fishery thus conforms to expectations based on girth measurements.

In pink salmon, by contrast, there is a sharp difference in the sexes. There were no females longer than about 450 mm but up to that length $\hat{s}_{ij}$ increased. Presumably, the 266-mm nets would catch at close to optimal efficiency for pinks beyond 450 mm. For males, peak $\hat{s}_{ij}$ values were close to 400 mm, suggesting that optimal efficiency was at a ratio of 1.0 rather than 1.2. The major difference in body shape and development of spawning characteristics probably accounts for the difference.

Summary comment on length–girth relations

Studies on length–girth relations and the relation between girth and mesh size have generally been indirect. Farran (1936) determined that maximum girth of herring varied with condition factor, which in turn determined whether fish of a given length would be captured in a mesh of given size. McCombie and Fry (1960) considered that maximum girth provided the best estimate of size of fish caught, but Regier and Robson (1966) raised the objection that girths had been estimated from lengths, and Regier (1969) concluded that unless precise measurement of girths could be taken at the mesh mark, it was better to continue to use length as the criterion for establishing selectivity.

In general, the minimum size fish that is retained is that for which maximum girth (at the leading edge of the dorsal fin in the case of salmon) approximates the perimeter mesh measure; the maximum size fish is that for which the girth at the posterior edge of the operculum (insertion of head with body) exceeds the perimeter mesh measure by some factor of body compressibility coupled with mesh elasticity (McCombie and Fry 1960; Regier and Robson 1966; Trent and Hassler 1968; McCombie and Berst 1969).

In this study, maximum girths were taken during the season, and some resampling was conducted several months later on fish that had been preserved by freezing for other purposes. These measurements demonstrated that, not only did maximum girths of all age class 1.3 sockeye exceed even the largest mesh size used in the experimental array, but so also did the girth taken just behind the operculum at the insertion of body with head.

The opportunity to compare catches and escapements was of great value in describing the selectivity of the Skeena fishery. For example, without supporting evidence from size characteristics of the escapement and catch, the suspicion that age class 1.3 sockeye were too large to have been gilled normally could not have been corroborated. Selectivity is usually determined only through the comparative fishing technique, and the shape of the selectivity curve is perforce assumed. Regier and Robson (1966) determined that a skew-normal model provided the best fit of several curve-fitting techniques for lake whitefish data; Holt (1963) used an unadjusted normal curve for data obtained from Fraser River sockeye; Olsen (1959) used an exponential model to obtain a satisfactory fit for Atlantic herring. For the direct approach, which has been applied to the Skeena sockeye and pink data, each $\hat{s}_{ij}$ simply represents the observed total rate of exploitation imposed by the fishery on each size class.

Discussion

The major findings of this study are threefold:

1. For the range of nylon mesh sizes from 4½ to 5½", the variability in the size of fish taken in any size mesh was sufficiently large that it was not possible to construct satisfactory selection curves. For age class 1.2 sockeye and pink salmon, both mean length and catches increased with mesh size. However, 1.3 sockeye, comprising three-quarters of the 1968 catch, were large in relation to all mesh sizes, and their capture was largely fortuitous. In consequence, in the aggregate, the selective effects of mesh size were masked.

2. Nylon nets caught about 2.5 times as many sockeye and 8 times as many pink salmon as comparable linen nets. The catches of the two kinds of nets were comparable with respect to age, sex, and length characteristics of the fish caught. Selective effects in the days of linen nets were probably similar to those of modern nylon nets.

3. Comparing catches and escapements for 1968, the fishery was shown to remove the smallest and largest sockeye at a relatively lower rate than those in a wide range of intermediate size. Selection is thus for the larger of the 1.2 sockeye, and the smaller of the 1.3 sockeye. Selection for pink salmon was for the larger fish, and was especially marked in females.

From these findings it may be surmised that the fishery has exerted, and could exert, selective effects on sockeye and pink stocks in years when there are substantial proportions of fish in the extreme size classes. In 1968, the year of study, the predominant 1.3 sockeye were among the largest recorded historically (Bilton et al. 1965, 1966, 1967, MS 1967, MS 1968), and the pink salmon were the smallest. In these circumstances, the fishery selectively removed the intermediate size sockeye and the larger pinks. More typically, it might be anticipated that the fishery would operate with fairly uniform effectiveness over most

of the upper range of sizes, with only the smallest pinks and 1.2 sockeye (and "jacks," 1.1 sockeye) being relatively underharvested. The long-term consequence of this pattern of selection is difficult to assess, for though it would tend to favor smaller fish in most years, it would occasionally be reversed. Bearing in mind the additional effect of periodic closure, which enables some of all size classes to escape the fishery, it seems reasonable to conclude that selective effects are not a major factor in the population biology of Skeena River sockeye and pink salmon.

Over the years the effectiveness of the Skeena River gillnet fleet has increased substantially. For instance, during the 1946–50 period, a fleet of between 800 and 900 vessels fishing 5 days per week caught between 50 and 60% of average size runs (Shepard and Withler 1958); during the 1960–68 period, a fleet of between 500 and 600 vessels fishing about 2 days per week also caught between 50 and 60% of average size runs. In terms of total relative efficiency, a gillnet fishing unit today is approximately four times as efficient as a unit of 20 years ago. A great part of the increased efficiency is due to the change in the gillnets.

A feature of the Skeena gillnet fishery, which tends to suggest that selection may not have altered substantially, even with the radical change in net materials, is that the fishermen have made an adjustment in the mesh size they use. Linen nets used in the river were of 5⅛-inch (143·mm) stretched mesh measure, whereas the nylon nets predominantly in use were found to be 5¼″ (133 mm). Catches recorded by the two nets in 1968 demonstrated the same age, size, and sex characteristics, but since the very large sockeye of age class 1.3 predominated in the run, it is likely that their influence masked any subtle differences between the two gear types that may exist.

Additionally, a feature that appears to be fairly unique to the Skeena River gillnet fishery is that the fishermen tend to use one size of gillnet regardless of changes in the size and age composition of the population, even between years. In a year such as 1968, this means that the largest fish tend to escape the fishery at a higher rate than would be expected if the nets were of a size to capture maximum weight. In other areas such as at Rivers Inlet, on the central British Columbia coast, fishermen usually carry two different mesh sizes so that a high efficiency of capture can be maintained regardless of the mean size or age composition of the run.

We conclude from this study that the Skeena gillnet fishery does not select from among the age and sex classes of sockeye and pinks in the same way from year to year, and therefore that it probably has not changed the productivity of escapements of given magnitude by altering their composition in some consistent direction over a long period. These conditions arise from the fact that both historically and currently the fishery does not respond to yearly fluctuations in size composition of the runs by concomitant changes in gillnet mesh size. Further, selection within any one fishing season is probably less than might be expected in view of the demonstrated ability of nylon nets to remove salmon selectively and their ability to remove a large proportion of fish present in a short time. The reason for this amelioration of selection appears to lie in the fact that the Skeena fishery is carried out mainly in continuous fishing periods of 2 or 3 days separated by periods of 4 or 5 days of no fishing, on fish that take an average of about 4 days to pass through the fishing area. Under these conditions, some groups of fish escape the fishing area virtually unfished in spite of an intensive selective removal of others.

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Zinc Retention in Young Rats Fed Increasing Levels of High-Zinc Oysters[1]

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SHAH, B. G., J. C. MERANGER, B. BELONJE, AND D. S. DESHMUKH. 1971. Zinc retention in young rats fed increasing levels of high-zinc oysters. J. Fish. Res. Bd. Canada 28: 843–848.

To determine the biological availability of zinc in oysters, 10 groups of 12 weanling rats each were fed diets containing 12, 60, 120, 240, and 2000 ppm added zinc either as zinc carbonate or as freeze-dried oysters. Four rats from each group were killed at 2, 4, and 8 weeks from the beginning of feeding, and their tissues analyzed. Neither the level of intake of zinc nor its source had a significant effect on body weight, hemoglobin and hematocrit, and liver cytochrome oxidase activity. At the 60-ppm level, the apparent absorption of zinc from oysters was 16.3 ± 3.7% (SE) and it was not significantly different from the corresponding value for $ZnCO_3$ (18.5 ± 2.2%). Zinc levels in soft tissues were not increased appreciably up to 260 ppm dietary zinc. At the 2000-ppm level, there was an initial increase that was not maintained for the duration of the experiment. Hair-zinc content did not vary with changing dietary intake but bone-zinc content increased with dietary level above 260 ppm and with duration of feeding at all levels tested.

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ZINC content of oysters has been reported by Schroeder et al. (1967) to be above 1000 ppm whereas most other foods average less than 30 ppm. Whether the large amount of zinc in oysters is biologically available is not known. In a recent review, O'Dell (1969) concluded that zinc in animal products (casein, egg white) is more readily absorbed than that of plant products (corn, soybean protein). To determine whether this general observation is applicable to zinc in oysters, at moderately low and high levels of intake, was the purpose of this experiment.

Materials and Methods

Ten groups of 12 weanling rats of the Wistar strain were fed the diets given in Table 1 ad libitum.

The oyster powder was prepared by freeze-drying oysters supplied by the Canadian Department of Fisheries and Forestry. The oysters were specially grown on zinc trays at Oyster Culture Station, Ellerslie, P.E.I. The dry powder contained (in ppm) Zn 11,800; Fe, 272; Cu, 94; Cd, 7; and Mn, 25. Equivalent amounts of these heavy metals were added to the zinc carbonate used in the diets.

Body weights of the rats and food consumption were recorded weekly. Four rats from each group were killed at 2, 4, and 8 weeks from the beginning of feeding, and blood, liver, kidney, muscle, femurs, and hair were collected for zinc analysis. The tissues were dry-ashed at 450 C and zinc determined by atomic absorption spectroscopy. Hair samples were extracted in a Soxhlet extractor for 2 hr with a 1:1 mixture of acetone and absolute ethanol, and then washed with demineralized water. After drying in the oven at 105 C the samples were digested with 4 ml of 50% nitric acid on a hot plate until dry and then placed in the furnace for ashing.

Hemoglobin and hematocrit were determined in the blood collected at 8 weeks, by the method described in Department of National Health and Welfare (1969).

Cytochrome oxidase activity was determined (Schnaitman et al. 1967) at 4 and 8 weeks in liver homogenates of rats, which were fed diets 1, 2, 9, and 10.

During part of the 7th and 8th week, food intake and fecal excretion of zinc were measured simultaneously for a period of 1 week in rats which were fed diets 3 and 4.

Results

The source of zinc or its dietary level had no significant effect on food consumption nor on the weight of the animals, which averaged from 381 ± 24 (SD) g to 406 ± 57 g for the various groups at the end of the experiment.

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TABLE 1. Composition of diets fed ad libitum to 12 Wistar strain rats for periods of 2, 4, or 8 weeks.

Ingredient	%	Ingredient	%
Vitamin-free casein[a]	20.00	$CaCO_3$	1.00
Corn oil	5.00	$CaH_4(PO_4)_2 \cdot H_2O$	1.26
Cellulose (Solka floc)[a]	2.00	Corn starch	25.00
Vitamin premix in alphacel[b]	1.00	Oyster powder or $ZnCO_3$[d]	
Mineral mix (excluding Zn)[c]	0.65	Sucrose	By difference

[a]Obtained from General Biochemicals, Inc., Chagrin Falls, Ohio.
[b]Deshmukh et al. (1970) except (g/kg mix) Ca pantothenate, 4.0; vitamin A powder, 25.0; *p*-aminobenzoic acid, 10.0.
[c]Deshmukh et al. (1970).

[d]Diet no.:	1	2	3	4	5	6	7	8	9	10
Oyster powder, %	.102		.510		1.019		2.038		16.983	
$ZnCO_3$, %		.0025		.012		.025		.049		.412
ppm Zn by analysis	22	25	70	78	132	149	259	289	1937	2362

Hemoglobin and hematocrit at the end of the experiment were not significantly affected by the dietary level of zinc nor by its source (Table 2).

The highest level of zinc from either source did not have any significant effect on liver cytochrome oxidase activity at 4 and 8 weeks (Table 3).

The apparent absorption of zinc from oyster powder and zinc carbonate at 70 and 78 ppm respectively was not statistically different (Table 4).

TABLE 2. Hemoglobin (Hb) and hematocrit levels (Hct) (avg ± SD) in rats after feeding on zinc carbonate or oyster powder diets for 8 weeks.

Zn in diet (*ppm*)	Hb (*g*%)	Hct (%)
22[a]	12.1±2.2	39.0±4.6
25[b]	13.4±1.0	42.0±1.2
70[a]	12.5±0.7	40.0±0.0
78[b]	11.8±1.1	36.2±3.6
132[a]	13.5±1.9	40.2±5.9
149[b]	14.0±0.7	43.0±1.2
259[a]	12.0±2.0	30.5±4.0
289[b]	13.1±2.6	39.0±8.0
1937[a]	12.6±1.4	41.2±2.2
2362[b]	14.2±2.8	42.8±1.0
Between levels:		
Oyster, F (4, 13)	0.416	0.270
$ZnCO_3$, F (4, 15)	2.038	2.123
P	N.S.[c]	N.S.

[a]Source of supplemental zinc oyster.
[b]$ZnCO_3$.
[c]Not significant.

Tissue levels of zinc are shown in Fig. 1–7.

Analysis of variance (Steel and Torrie 1960) of these results did not reveal any significant effect of the source of zinc but the dietary level had a significant ($P < .05$) effect on the concentration of zinc in all tissues examined except muscle and hair. The duration of feeding affected levels in all tissues except hair.

From Fig. 1 it is apparent that the source of zinc did not have any significant effect on blood zinc but the diet containing the maximum level of zinc caused a slight increase initially. There was an increase in blood zinc from 4 to 8 weeks, except at the highest level of intake.

At the dietary level of about 2000 ppm from either source the concentration of zinc in liver went down from 2 to 4 weeks (Fig. 2) whereas in muscle there was an increase from 2 to 4 weeks followed by a decline to the normal level (Fig. 3).

TABLE 3. Liver cytochrome oxidase levels (avg ± SD) (μ-at O_2/min per mg protein) in rats after 4 and 8 weeks.

Diet no.:	1	2	9	10
4 wk	.54±.03	.56±.01	.52±.06	.52±.10
8 wk	.55±.01	.49±.06	.56±.04	.46±.09

Source	df	F	*P*
Oyster	1,10	0.069	N.S.[a]
Time for oyster	1,10	1.141	N.S.
$ZnCO_3$	1,12	0.798	N.S.
Time for $ZnCO_3$	1,12	2.516	N.S.

[a]Not significant.

TABLE 4. Apparent absorption of zinc (avg ± SD) by rats from oyster powder and zinc carbonate.

Source:	Oysters	$ZnCO_3$	
No. rats:	7	8	*P*
	mg Zn/day		
Intake	1.345±0.101	1.533±0.107	<.01
Fecal excretion	1.125±0.151	1.246±0.088	N.S.[a]
Apparently absorbed	0.220±0.138	0.286±0.106	N.S.
	%		
Apparent absorption	16.3±9.9	18.5±6.2	N.S.

[a]Not significant.

Kidneys were not collected at 2 weeks, but the 4- and 8-week data shown in Fig. 4 indicate a similar trend at all dietary levels. It should be noted also that the 4-week values for groups 9 and 10 (about 2000 ppm) were higher than the corresponding values for the other dietary groups.

Levels of zinc in hair, surprisingly, did not show any consistent effect of either dietary intake or duration of feeding (Fig. 5). On the other hand the concentration of zinc in femurs (Fig. 6) increased with duration of feeding, but when the diet contained about 2000 ppm zinc the bone level dropped from 4 to 8 weeks by 30–40%. The total zinc in the femurs (Fig. 7) confirmed this trend. However, at the two higher levels of dietary zinc, the load of the element did not change appreciably from 4 to 8 weeks.

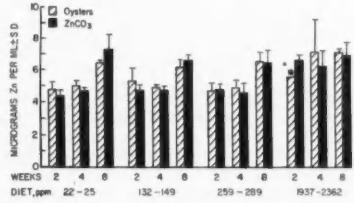

FIG. 1. Zinc levels in blood of rats fed zinc carbonate or high-zinc oyster powder diets for 2, 4, or 8 weeks. Diet groups 3 and 4 were excluded because the animals had to be killed 10 days before termination of the experiment on account of shortage of the two diets. Asterisk (*) indicates two observations.

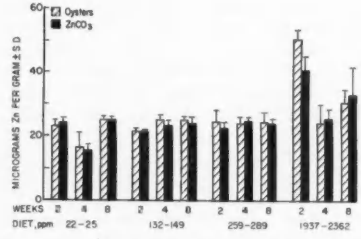

FIG. 2. Zinc levels in liver of rats fed zinc carbonate or high-zinc oyster powder diets for 2, 4, or 8 weeks.

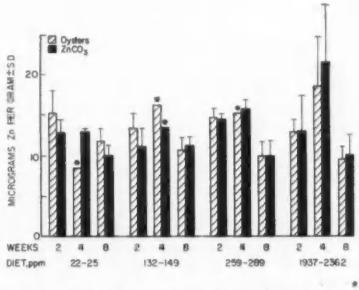

FIG. 3. Zinc levels in muscle of rats fed zinc carbonate or high-zinc oyster powder diets for 2, 4, or 8 weeks. Asterisk (*) indicates one observation.

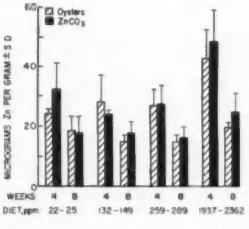

FIG. 4. Zinc levels in kidneys of rats fed zinc carbonate or high-zinc oyster powder diets for 4 or 8 weeks.

From these results it was apparent that the excess of zinc absorbed was laid down in the skeleton. It could, therefore, serve as an indicator of zinc retention. In Fig. 8 are shown the relative bone-zinc values calculated as follows: (1) total zinc in femurs was expressed as percent of total intake up to the time of killing (Table 5); (2) the value thus obtained was in turn expressed as a percentage of the value for the diet 1 (oysters) and diet 2 ($ZnCO_3$) to give the relative bone-zinc value; (3) the relative bone-zinc values for 2, 4, and 8 weeks were averaged separately for each diet and finally the means for the two sources, which were very close (diet 3, 33.0 ± 5.1; 4, 33.0 ± 0.7; 5, 18.7 ± 3.2; 6, 17.5 ± 2.5; 7, 9.7 ± 0.9; 8, 8.6 ± 0.5; 9, 2.9 ± 1.0; 10, 2.7 ± 1.0), were averaged for the four levels.

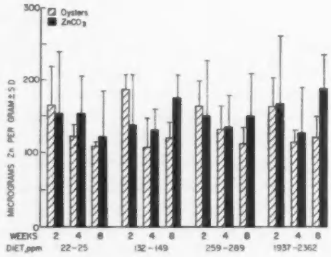

FIG. 5. Zinc levels in hair of rats fed zinc carbonate or high-zinc oyster powder diets for 2, 4, or 8 weeks.

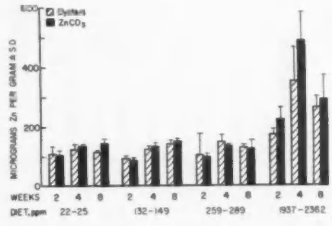

FIG. 6. Zinc levels in femurs of rats fed zinc carbonate or high-zinc oyster powder diets for 2, 4, or 8 weeks.

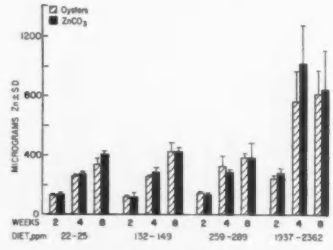

FIG. 7. Total zinc in femurs of rats fed zinc carbonate or high-zinc oyster powder diets for 2, 4, or 8 weeks.

The results in Table 5 indicate that at all levels of intake and from both sources the percent retention of zinc was reduced to half from 2 to 8 weeks. With an increase in dietary zinc from about 25 to 75 ppm, the relative bone-zinc value decreased from 100 to 33 (Fig. 8). Further increase to approximately 150 ppm caused the value to go down to about 18, and at 275 ppm zinc in the diet, the relative value was about 9. When the intake was at the maximum level of approximately 2000 ppm, however, the reduction in the relative value from 9.2 to 2.8 was not proportional. If the homeostatic control of intestinal absorption of zinc had operated efficiently at this dietary level, the relative bone zinc value would have dropped closer to one.

Discussion

The apparent absorption of zinc from oysters and from zinc carbonate at a dietary level of 75

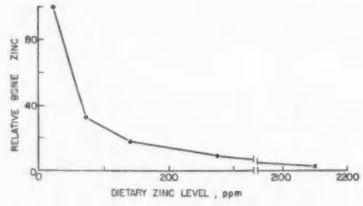

FIG. 8. Relative bone zinc as percent of intake; value for lowest level = 100.

TABLE 5. Fraction of dietary zinc (avg ± SD) in rat femurs (%).

Zn in diet (*ppm*)	Feeding period (*wk*) 2	4	8
22[a]	3.772±.540	2.728±.145	1.434±.103
25[b]	3.256±.382	2.601±.108	1.550±.126
70[a]	1.023±.159	0.987±.059	0.510±.034
78[b]	1.053±.119	0.880±.210	0.510±.066
132[a]	0.574±.075	0.526±.039	0.310±.055
149[b]	0.490±.083	0.458±.042	0.279±.035
259[a]	0.334±.014	0.292±.044	0.138±.012
289[b]	0.284±.030[c]	0.234±.016[c]	0.124±.022
1937[a]	0.075±.008	0.108±.023	0.038±.005
2362[b]	0.073±.008	0.102±.022	0.032±.010

[a]Source of supplemental zinc oyster.
[b]$ZnCO_3$.
[c]$P < .05$ for diets 7 and 8.

ppm was approximately equal (Table 4) indicating that there was no difference in the biological availability of the two sources of zinc.

Miller et al. (1970) reported comparable (19.3%) absorption of zinc from zinc oxide by normal young calves. The retention of an oral dose of ^{65}Zn 2–3 weeks after administration of the dose was reported to be 14% in normal rabbits (Graham and Telle 1967) indicating that the differences between the three species are not appreciable.

The blood hemoglobin, hematocrit contents (Table 2), and the cytochrome oxidase activity in liver homogenate (Table 3) were not significantly affected by the dietary treatments because the level of zinc fed was not as high as that used by Van Reen (1953) or by Settlemire and Matrone (1967a); or the copper content of the diets was high enough (21 ppm in diets 9 and 10) to obviate copper deficiency.

Blood zinc has been reported to increase from 60 to 154 μg/ml by Settlemire and Matrone (1967b) who fed rats a control diet with no zinc salt added to the salt mixture and an experimental diet having 0.75% of the metal. These values are 10–20 times higher than those observed in the present investigation (Fig. 1), which are in the range reported by Miller et al. (1970) in calves and by Bowen (1966) for mammalian blood. Blood zinc in groups 1–8 increased from 4 to 8 weeks, but this was not true in groups 9 and 10, indicating the presence of some homeostatic mechanism. The diets containing the highest level of zinc caused an initial increase in blood zinc that was in agreement with the observation of Miller et al. (1970) in calves.

Zinc levels in liver, muscle, and kidney (Fig. 2–4) of rats that were fed diets containing about 2000 ppm zinc clearly indicate that if the duration of the experiment is long enough the homeostatic mechanism can prevent the tissue content from remaining at the initially increased level. From their 21-day experiment on young calves, Miller et al. (1970) have concluded, however, that the homeostatic mechanism that limited zinc buildup in liver, pancreas, and kidney was overcome with a diet containing 633 ppm zinc. This may be due to the shorter duration of their experiment or due to a real difference between species. At the end of 5 weeks of feeding diets containing 0.5 and 0.75% zinc to adult female rats (Ketcheson et al. 1969) and to young rats (Settlemire and Matrone 1967a) homeostasis could not be maintained.

Although zinc in hair of pigs was reported to increase with higher level of dietary zinc (Lewis et al. 1957), no consistent effect of dietary zinc on the zinc content of washed rat hair was noted. Miller et al. (1970) reported a similar observation on zinc in the hair of calves.

From the increase in bone zinc with increasing dietary level it is apparent that excess of zinc absorbed is laid down in the bone of the rat. This is also true for pigs (Lewis et al. 1957) and for young calves (Miller et al. 1970). The load of zinc in bone is, however, controlled by a homeostatic mechanism (Fig. 8), probably acting on intestinal absorption because excretion of zinc through urine is minimal (Miller et al. 1970). The observations of Macapinlac et al. (1967) on squirrel monkeys and of Apgar (1968) on pregnant rats indicate that zinc in bone is not available to the animal when the dietary intake is deficient.

In conclusion, there was no difference between the availability of zinc from oysters and from zinc carbonate. The skeletal load of zinc in young rats increased at a dietary level of about 2000 ppm but not at 275 ppm. It has also been reported to increase at a level of 1550 ppm (Huber and Gershoff 1970) in adult rats. It is therefore probable that the homeostatic mechanism that regulates absorption of dietary zinc is overcome at a dietary level more than 275 but less than 1550 ppm.

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Sampling Benthos and Substrate Materials, Down to 50 microns in Size, in Shallow Streams

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Stream bed materials, both biotic and abiotic, in the size range 50 μ–ca. 200 mm can be sampled unselectively, in shallow streams, with a simple inexpensive apparatus consisting of a box provided with an adjustable upstream inlet, and, downstream, two nets, one within the other. Collected materials are wet-sieved and the volumes of inorganic material passed by successively finer sieves are plotted as cumulative curves against a logarithmic size scale. Curves are given for materials from three contrasting habitats: a riffle, and pool, of a coastal stream, and an artificial spawning channel. Examples are also given of the densities, size distribution, and vertical stratification of invertebrates from these habitats. Applications of the method to studies on fish biology, invertebrate ecology, and geomorphology are indicated.

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THE materials that lie on the bed of a stream consist of inorganic items, such as clay and cobbles, organic detritus, and living items, such as insects and fish eggs. Irrespective of whether they are biotic or abiotic and irrespective of their densities, many of them can be regarded simply as particles of different sizes. Three groups of workers are concerned with sampling some of these materials unselectively. The fish biologist is interested in sampling eggs and alevins in spawning beds, and in assessment of silt because of its effect on their survival. The invertebrate zoologist samples streams primarily to determine invertebrate density and production, and is interested in particle-size characteristics of the substrate as they affect organisms. The hydraulic engineer and the fluvial geomorphologist measure rugosity of natural channels and use particle-size distributions of bed materials to interpret river mechanics.

Certain basic conditions of obtaining an unbiased sample seem to apply. Firstly, it is frequently impossible to push any apparatus into the stream bed, and if one succeeds, one is disturbing and dispersing material (silts, clays, and organisms) one is trying to collect. Secondly, if anything is placed on the stream bed it must cause no downward displacement of water that would disperse particles; also, it must be streamlined in form so that there is minimum resistance to flow past it. Finally, if a fine net is used to collect silts, the flow into it must be controllable so that a backwash is not generated, with consequent loss of materials.

Some techniques currently used for sampling stream benthos meet these requirements only in part (for reviews or discussions of these see, for example, Macan 1958, Cummins 1962, Southwood 1966, and Ulfstrand 1968). These aim at assessing the numbers of individuals, or biomass, of invertebrates per unit area of substrate and they delimit an area of stream bed within which the materials are disturbed;[1] the fauna is thereby dislodged into the current and so carried into a collecting net. This is the principle of samplers described by Surber (1937), Hess (1941), Waters and Knapp (1961), and Allen (1940). The Hess and Waters and Knapp cylindrical samplers are pushed into the stream bed, which limits their use to substrates with small or medium-size stones (cf. Ulfstrand 1968 p. 38). All these samplers employ a net and so impose a mesh selection on the animals collected. If the net has a mesh of, say, 350 μ or more, most of the invertebrates will pass through it; if a mesh is chosen that will retain the smallest instars of insects, then it clogs rapidly, the current through it is impeded, a backwash builds up, and the fauna is again inadequately sampled (cf. Macan 1958 p. 17; Ulfstrand 1968 p. 38).

[1]Cummins (1962) stresses the need to remove the inorganic materials along with the fauna.

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To reduce these sources of error an inexpensive sampler was designed.[2] It is not worked into the stream bed, the inflowing current can be controlled, and the animals and substrate materials are collected unselectively between 50 μ in least diameter and 200 mm or occasionally more. Within certain limitations (stated below) it therefore largely, but not entirely, meets the requirements of the geomorphologist, fish biologist, and invertebrate zoologist. The last, moreover, can investigate some factors that are major determinants of faunal densities, i.e., the particle size distribution and compactness of the substrate materials (Cummins 1962; Cummins and Lauff 1969), the amount of allochthonous plant detritus that can serve as food (Egglishaw 1964), the algal densities on the superficial stones (see discussion in Ulfstrand 1967), and the velocities and patterns of flow on these as they affect the distribution of insects that extract foods from the current, e.g., Simuliidae, Hydropsychidae.

The Sampler

Essentially the sampler is a floorless box 45 cm high (choice of dimensions is not critical) and 45 cm wide with a narrow vertical upstream inlet, and downstream, two collecting nets, one within the other (Fig. 1, 2*a*, *b*). The area of substrate enclosed is 0.18 m². The box is made of 12-mm (0.5-inch) water-proofed plywood; the converging portions of the sides are secured to the parallel sides by duralumin strips held by screws (*c*); rigidity is obtained by wooden bars across the top (*b* 2) and by a single bar, oval in section, between the converging sides (*a* 1; *b* 1). A length of curved aluminum sheet is fitted to the inlet, and is held in position by a piece of elastic tubing (Fig. 1, 2*e*). The box has a half back of plywood (*a* 2). Angle duralumin (*f*) is screwed to the sides at the back and also extends across the base; this receives the canvas-covered net frames, which are of brass wire, with the inner fitting snugly inside the outer. The canvas of the nets is about 15 cm wide (*a* 3, stippled). The inner net is a bag 55 cm long, made of three pieces of "Nitex"[3] cloth (a top, a bottom, and a continuous piece joining these) of mesh size 600 μ (30 meshes per liner inch); the outer net is made of four pieces (i.e., it is pyramidal) of 50 μ "Nitex." It is 100 cm long and tapers to a canvas sleeve. The seams of the net are strengthened by nylon tape. A canvas sleeve at the apex accepts a plastic collar secured by a hose clamp. The collar is threaded internally and a plastic collecting bucket is screwed into it (*d*). This has two large holes in its walls; these are covered with 50 μ "Nitex."

The sampler is placed on the stream bed *with the nets in place* so that the stream-lined shape

[2]A preliminary account was presented as a summary note in the Proceedings of the 50th annual conference of the Western Association of State Game and Fish Commissioners and the Western Division of the American Fisheries Society.

[3]"Nitex": trademark of Tobler, Ernst and Traber, Inc., New York, N.Y.

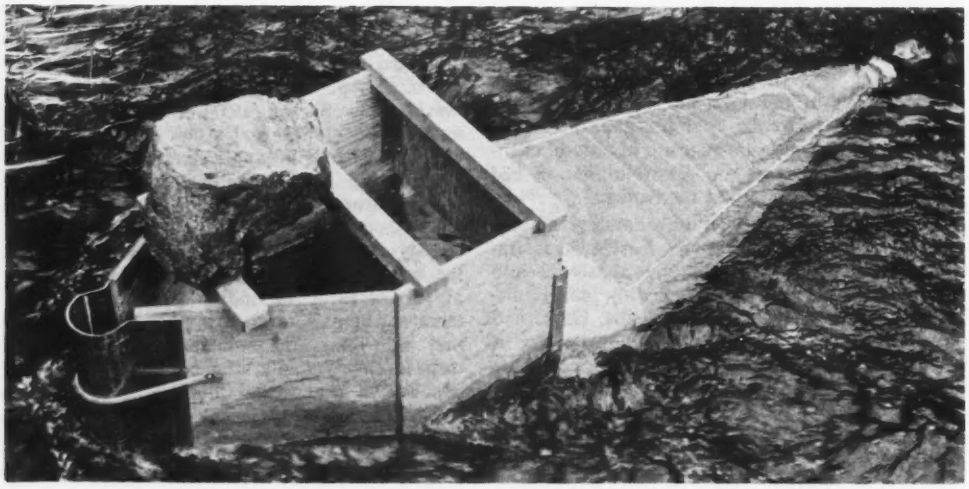

FIG. 1. Stream sampler in use.

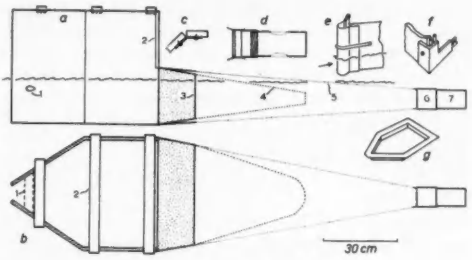

FIG. 2. Constructional details of sampler. *a*, side view; *b*, plan (the scale applies to *a* and *b* only); *c*, method of securing wooden panels together; *d*, collecting bucket; *e*, three-dimensional diagram of front of sampler in use; *f*, three-dimensional diagram of rear corner showing method of securing the net frames; *g*, three-dimensional diagram of frame for substrate materials.

of the total apparatus causes a minimum of turbulence. The forward end is lowered into the water first. Localities with one or more conspicuously obtruding large stones (>20 cm) are avoided. A shoulder of gravel is then built up around the outside if the site is a riffle, but may not be required if the site is a pool with sandy substrate. Stones are picked up downstream of the sampler, and the shoulder serves to reduce or occlude the flow of water under the side into the sampling area. This appears to make a more effective seal than a continuous strip of foam rubber around the base (Warren et al. 1964 p. 645). Loss of material below the crossbar (Fig. 2*f*) is unlikely unless the net is clogging (see below), but can be prevented by application of gravel behind the bar from outside, or of pieces of foam rubber (15 × 5 × 5 cm) below the bar from inside.

The current entering the sampler by the vertical inlet is controlled by raising or lowering the aluminum inlet cover until an adequate flow is obtained. With the flow restricted there is little turbulence within the sampler, so that the substrate becomes clearly visible through the water. When the sample is being taken, therefore, and stones are being removed or disturbed, it is possible to observe the silt particles being carried into the net and to note whether any are passing below the crossbar. Any material entering the box from beneath the sides can also be seen. (An alternative inlet cover of two short lengths of plate separated by a gap caused the water to enter some 15 cm above the substrate; no advantage resulted.)

If one person is operating the sampler, it may be necessary to anchor it with a large stone placed on the forward crossbars (Fig. 1). When a sample is taken, the cobbles, gravel, and coarse sand within the box are lifted out by hand or with a trowel. Cobbles are brushed and washed so that adhering invertebrates are carried into the net. The large inorganic materials are either transferred to a container and retained for physical analysis, or they are discarded. The remaining gravel, sand, and silts are conveyed into the net with the trowel rather than stirred up. The object is to transfer the substrate materials to the nets. In situations such as artificial spawning channels, sands may be most abundant near the surface of the bed and disturbance might allow them to settle further into the gravel. This would not occur with the lighter silts that come into suspension and are carried into the net.

The sampler works best in water less than 30 cm deep, but is usable in slightly deeper water. A higher sampler would be feasible, but the need to be able to reach to the bottom imposes a limit.

The depth of substrate from which materials can be removed varies with the substrate. A common workable depth in stream riffles is 20–30 cm, but in pools, where the substrate is mainly sand, 15 cm may be the limit. The tendency for material to slump into the sampling area increases as the depth increases. Materials are removed to leave, as far as possible, a hole with vertical sides of regular depth. In practice the hole is likely to be irregular, and judgment must be used in deciding which stones near the sides should be removed and which left. The irregularity is a source of imprecision.

The coarse inner net retains the larger animals and materials that have been carried into it, and protects the fine net that retains items down to 50 μ in least diameter. This net must be protected from abrasion from stones.

If it is of interest to record the vertical stratification of materials or invertebrates, the nets are replaced by new ones as the sampling depth increases. During the exchange of nets the opening into the sampler is occluded by the plate being pushed into contact with the substrate. Complete occlusion may not be possible.

If the movement of suspended particles suggests that the fine net is clogging and some backwash is taking place, new nets must be used. Flow through the nets is facilitated if the outside of the fine net is wiped to dislodge adhering particles (this must cause some loss of particles 50 μ in size), and if stones obtruding from the stream bed below the net are removed so that the collecting bucket is not elevated.

An additional application of the sampler is its use in conjunction with an angle-iron frame that

delimits a colonizing area containing either a natural substrate or one of particles of a selected size (e.g., sand or gravel). Clean materials, surrounded by the frame (Fig. 2*g*, 3), are left in the stream for 1 week or more (Ulfstrand 1968 p. 100) so that they can be colonized by fauna. The frame has a shape corresponding to the base of the sampler, which can be placed on top of it and the invertebrates can be collected.

The cost of materials, including a spare fine net, and construction of the sampler is about $80.

Physical Analysis

Methods

The smallest particles collected (50 μ) are in the middle of the coarse-silt category on the Wentworth scale (e.g., King 1966) or the scale of the American Geophysical Union (e.g., Einstein 1964). Medium silts, fine silts, and all clays are lost. This is a limitation of the method.

The absence of very fine particles permits a uniform treatment of the inorganic materials during mechanical analysis, i.e., particles of different sizes can be separated by the same means. After manual removal from the sample, items over, say, 32 mm in minimum diameter are placed in geometrically increasing size categories and their volumes determined by displacement.

Fig. 3. Frame surrounding substrate materials in stream.

The remainder of the sample is wet-sieved, and the amounts retained on each of a series of sieves can be established as a weight or a volume. Although the measurement of volumes is less accurate than that of oven dry weights, volumes are likely to have more ecological significance and are independent of specific gravities (Morgans 1956; Cummins 1962). Volumes were therefore measured after the sieve residues had settled in water in standard cylinders for a standard time (12 hr).

Collection of a sample in the field takes about 30 min; its physical analysis (apart from 12 hr settling time) takes about 8 hr.

Results

The results are best presented in the form of cumulative curves related to a logarithmic size scale, i.e., the cumulative curve shows the percentage, by volume, of the sample consisting of particles smaller than a given size. A useful logarithmic scale is provided by phi units where phi (φ) is $-\log_2$ of the diameter in millimeters. Thus the arithmetically unequal intervals of the Wentworth scale are converted into equal intervals on the φ scale and the cumulative curve of an analysis can be plotted on equal-interval graph paper along the abscissa without changing the shape of the curve (for discussions on methods of presenting results of analysis see Inman 1952; Morgans 1956; King 1966). Here, particles of smallest size are placed to the left. Both equal-interval scales and arithmetic-probability scales are used to illustrate the examples. King (1966) discusses the advantages of the probability scale and the values used in describing a particle-size distribution.

The most useful descriptive measure of the curves, for the biologist, may be considered. One measure of the central tendency is the median diameter, or the 50% value on the cumulative curve. This can be misleading as it takes no account of the distribution of grain size on each side of the 50% value. Another, the mean, is defined by Inman (1952) as $M\varphi = 1/2\ (\varphi 16 + \varphi 84)$, but this also is unsatisfactory for polymodal distributions (see King 1966). It seems simplest and most instructive to establish from the curves those data likely to be of ecological significance. For example, the percentage by volume of the material of size 1 mm and less can be stated. This is an arbitrary but convenient measure of the fines of the fish biologist. The percentage of material of size 2–6 cm may also be stated, as it is an arbitrary size range of much good spawning gravel. These two measures are used in the examples. The percentage of other size categories can, of course, be read from the curves.

Example 1

Figure 4 shows the curve obtained from the volumetric analysis of inorganic materials taken with the sampler from a riffle of a small creek. The water depth at the site was 15 cm and the surface velocity 60 cm/sec. The substrate appeared to be suitable for spawning salmon. The sample extended 32 cm into it. Material of 1 mm or less amounted to 7% of the total sample, and material in the size range 2–6 cm amounted to 34%.

Example 2

Figure 5 shows, in contrast to the previous sample, the curve of inert materials taken from a pool (depth 21 cm, surface velocity 15 cm/sec) a short distance downstream of the riffle of example 1. The sample reached a depth of 25 cm. Materials of 1 mm or less made up 41%; materials in the size range 2–6 cm made up 15%.

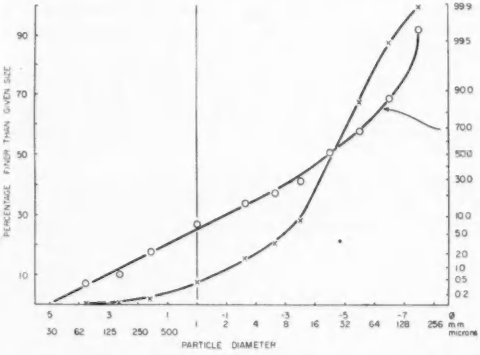

FIG. 4. Cumulative frequency curves of volumes of inorganic materials from a riffle; size scales are in phi units, millimeters, and microns.

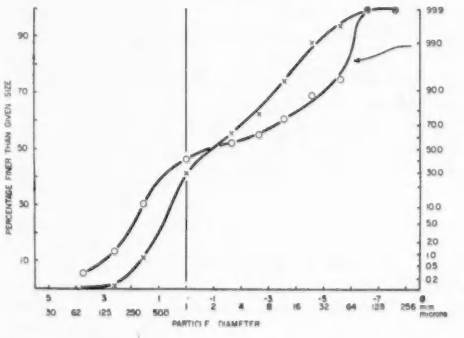

FIG. 5. Cumulative frequency curve of inorganic materials from a pool.

Example 3

Figure 6 shows the curve for inert materials in an artificial spawning channel. The water depth of the sampling site was 35 cm, the velocity 75 cm/sec, and the sample reached a depth of 25 cm. In the sample examined materials 1 mm or less amounted to 2% of the total volume; materials of 2–6 cm amounted to 60%.

Biological Analysis

METHODS

The objectives of faunal analysis are to estimate the numbers of invertebrates in the samples, to measure their size distribution, and to establish taxonomic categories and percentage composition.

When a sample is collected the larger stones are picked off the stream bed and brushed, within the sampler. The coarse net in the apparatus is used only to protect the fine net. Its contents are added, in due course, to those of the fine net. The benthic materials are transferred to wide-necked polyethylene jars and formalin is added. If vertical stratification is to be examined, the nets are replaced after the top 10 cm of materials have been sampled. If only the fauna is of interest the cobbles and gravel are discarded. Where moss and filamentous algae are present they would justify being kept separate from inorganic materials and being examined for their associated fauna.

The sieving procedure for physical analysis (carried out in the laboratory) is also the basis for extracting and counting the animals. When the sample is washed on the 1-mm mesh sieve (16 meshes/inch) the residue is examined, firstly with the unaided eye, and secondly with a 12× stereomicroscope, and the fauna is picked out. All fragments of leaves, twigs, insect cases, and

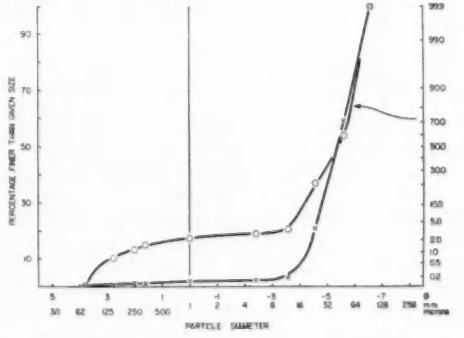

FIG. 6. Cumulative frequency curve of inorganic materials from an artificial spawning channel.

debris are examined and removed. Since there is no subsampling of the animals retained on this sieve, this step in the procedure determines that, whatever treatment is given to the animals retained on subsequent sieves, the counts have to be related to the total sample and not to an aliquot of it.

The filtrate of the 1-mm mesh sieve is washed on a 350-μ sieve, the filtrate of this on a 250-μ sieve, of this on a 150-μ sieve, of this on a 74-μ sieve, and of this on a 50-μ sieve. The residues on these sieves are then subdivided frequently to reduce the time required to count the animals present. Each residue is transferred to a 1000-ml beaker to which water is added to make the contents up to about 400 ml. Into a second beaker of the same size are placed as many upright thin-walled vials as possible. The vials used were 5 cm in height and 1.5 cm in diameter, and the beaker accepted 22; the bundle was held together by a rubber band. About 500 ml of water are added so that the vials are just submerged. The sample in the first beaker is stirred to bring it into suspension and is poured into the second beaker. If a residue is large it may be necessary to treat it in two halves in this way. After the materials have settled the water is siphoned off to a level just below the top of the vials. The band is cut and the vials are lifted out individually and arranged in a row. Those containing about average heights of material are selected; their contents are transferred to small petri dishes, and the animals are counted under a stereomicroscope capable of 50× magnification (the discrimination of small stages of some species requires the preparation of mounts). The area of the mouth of one vial was 1/45 of the cross-sectional area of the beaker so a count of animals from one or more vials could be related to the total material. The principle of subdividing samples by making them settle in a group of containers has also been used by Waters (1969). Its use here is facilitated by the homogeneity of the material resulting from the sieving.

Flotation of the animals in the vials with sugar solution did not prove advantageous, as much of the silt also became buoyant.

The usability of the settling procedure for estimating the total number of invertebrates is dependent upon the animals settling into the vials at random. Even, however, if the variance of the counts exceeds the Poisson variance on occasion, the method may still give adequate accuracy. Tables 1*a* and *b* give results from two series of sieve residues and *c* gives results of treating two filtrates of the 350-μ sieve. This second procedure is appropriate where particle-size analysis of the inorganic materials of a sample is not required; it is then possible to give a single treatment to all the material that has passed through the 350-μ sieve.

Table 1 shows that the counts tend to conform to random distributions as tested by X^2. In a Poisson distribution the accuracy of a count will be determined solely by its size. The degree of accuracy can be expressed

TABLE 1. Counts of invertebrates in sets of settling tubes; (*a*) and (*b*) are series in which the materials were washed on four sieves; (*c*) and (*d*) are series in which the materials had passed through only one coarse sieve. The counts are not significantly different from random at the 0.95 probability level, except in the (*c*) series where one count (58) causes departure from randomness.

Residue on sieve of mesh (μ)						$\bar{m}$	X^2
				(*a*)			
350	21	34	24	31	31	28	4.2
250	48	43	35	37	46	42	3.0
149	44	41	46	41	43	43	0.4
74	13	9	11	12	8	11	1.8
				(*b*)			
350	39	40	49	56	58	48	6.4
250	35	34	44	39	45	39	2.6
149	86	98	82	75	73	83	4.8
74	25	18	28	25	14	22	6.0

											$\bar{m}$	X^2
						(*c*)						
Total filtrate through sieve of mesh 350 μ	100	113	70	107	58	103	99	91	101	89	93	28.1
						(*d*)						
Total filtrate through sieve of mesh 350 μ	65	87	85	97	89	91	89	109	84	93	74	79	$\bar{m}$ 87	X^2 15.8

by the standard error as a percentage of the count. At 0.95 confidence limits a count of 100 will fall between 80 and 120; a count of 196 will fall between 168 and 224. The desired accuracy must be chosen and the contents of vials examined until a large enough sample size has accumulated. It is not sound practice to examine a fixed number of vials irrespective of their contents (for a discussion of estimating soil fauna see Jones 1955). If animals are, in fact, rare, a count of 100 is not possible, so a high degree of accuracy is unobtainable. Additional field samples would be required to give an accurate estimate of these.

The manner of sieving is important. Animals can be forced through sieves with jets of water, and are frequently fragmented in this way. The correct washing procedure is to agitate the sieve vertically in a large volume of standing water.

Results

Example 4

A sample taken on July 29 from the riffle examined in example 1 gave an estimated 4378 invertebrates (24,079/m²); 62 distinguishable species were present. Their size distribution (head widths) is shown in Fig. 7 as a histogram and as a cumulative percent frequency curve. It is seen that 80% of the animals have head widths of 250 μ or less; most of these are chironomids. Individual species of insects do not necessarily have their greatest numbers in the smallest size category. For example, a species of *Hydropsyche* in the sample ranged from 120 to 840 μ in head width but the mode was conspicuously at 240 μ.[4] Table 2 shows the percent composition of the taxa in the sample.

Example 5

Another sample from the riffle treated in example 1 was taken on April 15. The fauna of the top 10 cm was collected separately from that between 10 and 32 cm. The total number of invertebrates in the top layer was 5739 and in the lower layer 2400 (total number of all animals amounts to 44,765/m²). Table 2 shows that the Simuliidae account for the most obvious difference in the two layers, but other differences occur. These invertebrates were obtained from the materials analyzed physically in example 1.

Example 6

The fauna of the materials obtained in April from the pool of example 2 comprised 4903 animals (26,966/m²); 3927 invertebrates occurred in the top 10 cm and 967 between 10 and 25 cm. The proportions of Simuliidae, mayfly nymphs, and chironomid larvae are greatly different from that of example 5, and this is expected in view of the physical differences of the habitats (Fig. 4 and 5). Detailed differences are shown in Table 2.

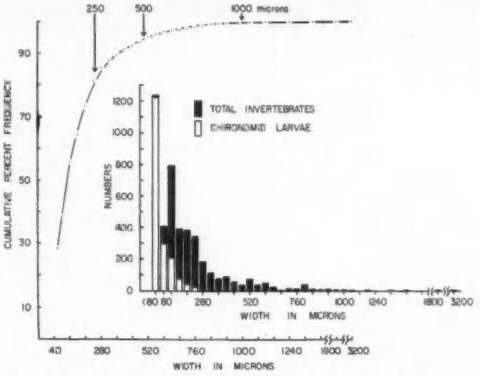

FIG. 7. The size distribution (as head widths) of insects from a riffle, expressed as a histogram (inner figure) and as a percentage cumulative frequency curve (outer figure); arrows point to sizes of meshes of sieves commonly chosen for washing benthos.

Example 7

The fauna of the materials analyzed in example 3 comprised 10,413 animals (57,271/m²) of which 7386 were in the uppermost 10 cm and 3027 were between 10 and 25 cm. Table 2 gives the compositions. The high density is presumably attributable to the low silt content, negligible sand content, high intragravel flows, and to the blanket of filamentous algae that develops on strongly lit surface gravel. This harbors a *Cricotopus* (Orthocladiinae) fauna.

Discussion

Limitations of the Method

One limitation is the depth of water in which the sampler can be used. Geomorphologists sometimes obtain samples when bed materials are temporarily exposed at river margins.

Artificial spawning channels may be slightly too deep in which to operate the apparatus, unless the water level can be temporarily lowered. The collection of fines only, however, is required from artificial channels, as the particle-size distribution of the gravel is known. It might therefore be possible to work with a high-sided sampler and to disturb the gravel with a long-handled trowel.

[4]An obvious cause of polymodal size distributions would be overlapping of generations of a species.

TABLE 2. Numbers and percentage composition of the commoner invertebrates collected. Top refers to the uppermost 10 cm of substrate, bottom to depths below 10 cm. A = adult, P = pupae. See text for descriptions of localities.

	Example 4 Riffle July 29	Example 5 Riffle April 15		Example 6 Pool April 15		Example 7 Spawning channel July 14	
	Total	Top	Bottom	Top	Bottom	Top	Bottom
No. per sample	4378	5739	2400	3927	976	7386	3027
No. per m²	24079	31564	13200	21598	5368	40623	16648
Collembola							
Folsomia sp.	–	–	–	–	–	–	<0.5
Smynthurides sp.	–	–	<0.5	<0.5	–	–	–
Ephemeroptera							
Baetis parvus Dodds	6.6	<0.5	<0.5	2.6	1.8	<0.5	<0.5
Baetis intermedius Dodds	7.8	3.1	<0.5	<0.5	<0.5	<0.5	–
Ameletus sp.	–	–	–	0.5	<0.5	–	–
Ephemerella inermis Eaton	–	<0.5	–	<0.5	<0.5	–	<0.5
Ephemerella spp.	1.5	0.5	<0.5	–	–	<0.5	<0.5
Paraleptophlebia sp.	0.9	–	<0.5	5.5	9.5	0.6	1.3
Iron sp.	–	1.6	<0.5	–	–	–	–
Rithrogena sp.	11.6	<0.5	<0.5	–	–	–	–
Cinygmula sp.	0.9	1.1	<0.5	2.4	2.3	–	–
Cinygma sp.	<0.5	–	–	–	–	–	<0.5
Plecoptera							
Chloroperlinae	1.0	3.8	2.2	2.3	3.6	0.9	<0.5
Isoperla spp.	<0.5	4.0	6.6	7.9	9.3	–	<0.5
Nemoura spp.	<0.5	–	–	–	–	11.8	7.2
Trichoptera							
Hydropsyche sp. 1	–	–	–	–	–	4.6	1.6
sp. 2	–	–	–	–	–	3.4	0.8
sp. (1?)	4.3	0.8	<0.5	<0.5	–	–	–
sp. 2 (P)	–	–	–	–	–	–	<0.5
Cheumatopsyche sp.	–	–	–	–	–	1.5	0.7
Glossosoma sp.	8.8	<0.5	<0.5	<0.5	–	–	–
Wormaldia sp.	–	–	–	–	–	<0.5	<0.5
Rhyacophila spp.	<0.5	<0.5	<0.5	<0.5	–	<0.5	–
Limnephilidae spp.	<0.5	<0.5	<0.5	1.6	1.5	<0.5	–
Oxyethira sp. (P)	–	–	–	–	–	–	<0.5
Other Hydroptilidae	–	–	–	–	–	–	<0.5
Other Hydroptilidae (P)	–	–	–	–	–	–	<0.5
Diptera							
Tanypodinae	0.9	<0.5	1.8	13.2	10.7	9.7	13.0
Diamesinae	<0.5	–	–	–	–	–	–
Orthocladiinae	16.8	9.1	16.2	20.3	13.1	43.2	49.8
Orthocladiinae (P)	–	<0.5	<0.5	<0.5	<0.5	1.0	–
Corynoneura spp.	3.2	3.6	8.0	20.5	11.5	2.6	6.3
Thienemanniella spp.	3.3	2.7	3.6	5.1	7.9	0.9	–
Chironomini	3.2	16.6	34.2	0.5	–	3.3	–
Tanytarsini	11.0	1.0	2.0	11.9	20.7	4.4	4.7
Tanytarsini (P)	–	–	<0.5	–	–	–	–
Ceratopogonidae	0.5	<0.5	2.1	<0.5	<0.5	–	–
Antocha sp.	<0.5	<0.5	–	–	–	–	<0.5

(*Continued*)

TABLE 2. Numbers and percentage composition of the commoner invertebrates collected. Top refers to the uppermost 10 cm of substrate, bottom to depths below 10 cm. A = adult, P = pupae. See text for descriptions of localities. (*Concluded*)

	Example 4 Riffle July 29	Example 5 Riffle April 15		Example 6 Pool April 15		Example 7 Spawning channel July 14	
	Total	Top	Bottom	Top	Bottom	Top	Bottom
Hexatoma sp.	<0.5	<0.5	<0.5	–	–	–	–
Limnophila sp.	–	–	<0.5	–	–	–	–
Other Tipulidae	<0.5	–	<0.5	<0.5	<0.5	–	–
Tipulidae (P)	–	<0.5	–	–	–	–	–
Prosimulium sp.	<0.5	34.8	0.7	<0.5	–	–	–
Prosimulium sp. (P)	–	0.8	–	–	–	–	–
Simulium spp.	–	10.6	<0.5	–	–	2.3	<0.5
Dixidae	–	–	–	–	–	<0.5	–
Blepharoceridae	–	<0.5	<0.5	–	–	–	–
Chrysops sp.	–	–	–	<0.5	–	–	–
Empididae	<0.5	<0.5	–	–	–	<0.5	<0.5
Coleoptera							
Zaitzevia sp. (A)	<0.5	<0.5	–	–	–	–	–
Zaitzevia spp.	2.1	1.3	3.9	<0.5	<0.5	–	–
Narpus sp.	0.7	<0.5	<0.5	–	–	–	–
Oligochaeta							
Nais spp.	<0.5	<0.5	1.4	<0.5	–	<0.5	2.0
Pristina sp.	<0.5	–	–	–	4.1	<0.5	–
Slavina sp.	–	–	–	–	–	1.0	1.0
Lumbriculus sp.	0.8	<0.5	<0.5	–	–	<0.5	<0.5
Eisienella sp.	–	–	–	–	–	–	<0.5
Other Oligochaeta	–	<0.5	0.6	–	–	–	–
Turbellaria							
Cura foremanii (Girard)	<0.5	–	<0.5	–	–	0.7	3.6
Phagocata sp.	–	–	–	<0.5	–	–	–
Acari							
Torrenticola spp.	2.3	<0.5	7.0	<0.5	<0.5	<0.5	<0.5
Unionicola sp.	–	<0.5	–	–	–	–	–
Ljania sp.	<0.5	<0.5	<0.5	<0.5	–	–	–
Hygrobates sp.	–	–	<0.5	–	–	–	–
Lebertia sp.	–	–	–	<0.5	–	–	–
Tyrrellia sp.	–	–	–	–	–	<0.5	<0.5
Sperchon spp.	<0.5	<0.5	–	–	–	–	–
Hydrozoetes sp.	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Malaconothrus sp.	4.6	<0.5	–	–	–	–	<0.5
Trimalaconothrus sp.	1.0	–	<0.5	–	–	–	<0.5
Mollusca							
Physa sp.	–	–	–	–	<0.5	0.6	1.1
Planorbidae	–	–	–	<0.5	–	–	<0.5
Sphaeriidae	–	–	–	<0.5	<0.5	–	<0.5

Another limitation is the depth into the substrate to which sampling can penetrate. This might be increased if the sides of the box were extended downwards as the sample is being taken. Metal brackets could be fitted to the inside to accept pieces of wood; these could be lowered to prevent slumping of materials into the sampling area. They would be useful when materials were being

taken from pools, but they could not be operated if large stones were present.

The area of substrate sampled is constant, but the volume of materials removed varies with depth and irregularity of the cavity. This must be taken into account in comparisons of samples or strata.

The total loss of fine silts and clays is an irreducible limitation. The fine net could be made slightly finer, say, to 30 μ mesh size, but this is well outside the clay range. Furthermore, clogging difficulties would occur. Perhaps the assessment of fine silt and clay might be approached by attempting to relate bed materials to suspended load, a subject about which little is known. If fine silts and clay occur in the absence of gravel and cobbles they can, of course, be sampled with corers.

Applications

Fish biologists are concerned with assessing silt and describing quantitatively the physical characteristics of spawning bed materials. Various methods have been described that involve working a device into the substrate (Hatch 1957; McNeil and Ahnell 1964; Rukhlov 1969; Ryan 1970), but have the drawback of causing losses of the silts. The measurement of materials down to 50 μ in size is probably adequate for describing the characteristics of habitats that sustain salmon. (Situations could exist where an exceptional geology or man's influence introduced to a stream bed large quantities of clay; the sampler could not assess these.) Artificial spawning channels have exceptional particle-size characteristics. As they age they accumulate silt and it becomes necessary to clean them. There is need for detailed studies relating the field measurement of fines to fry production. The influence of filamentous algae that develop as extensive mats on the gravel is insufficiently examined, as is the possible role of invertebrates as predators of fish eggs and alevins. The sampler could be used for study of these problems.

A well-tried method for recovering salmon eggs and alevins from gravel exists (McNeil 1964), but has drawbacks in coarse gravels (Merrell 1965). The present sampler should be effective for collecting eggs, provided a single coarse net is used. It is unlikely, however, that the sampler would be useful for collecting alevins, which would quickly move through the gravel. At most, if used promptly, with a net of appropriate mesh, it might provide an index of alevin density.

Living invertebrates can pass through meshes that are smaller than their maximum body width; in dipterous larvae, for example, the diameter of the head capsule determines whether a larva will be retained or not (Jonasson 1955). In general the maximum widths of head capsule of common stream invertebrates fall between 60 μ for newly hatched chironomid larvae to about 5 mm for large Plecoptera (much larger aquatic insects exist but they are not abundant). The younger stages tend to predominate in a natural community. Consequently, even a mesh of 116 μ (Anderson 1967) could allow 50% of the fauna to pass through if the community contained high proportions of chironomid larvae, mayfly nymphs, and stonefly nymphs. The sampler can, of course, be used with a single net of 200- or 250-μ mesh. This would allow perhaps 70–80% of the animals to pass through, but would be adequate for many purposes, such as the estimation of biomass and for general faunistic surveys.

A source of inaccuracy in estimating stream invertebrates may be the depth to which the fauna penetrates. Most samplers retrieve animals from the top 10 to 15 cm of the substrate only, but significant numbers may live below this. Evidence that invertebrates may occur down to 30 cm or more has been obtained (Coleman and Hynes 1970) from the colonization, up to a period of 28 days, of sand and gravel placed in perforated cylinders and buried in the substrate. That higher flows through the substrate than those in the undisturbed state may have occurred was suggested by the initial presence of *Simulium* below 22 cm and by the continued presence of Hydropsychidae (cf. example 4). There seems no reason to doubt that wherever adequate intragravel flows occur invertebrates will be found. Experimental studies show that a clean homogeneous gravel bed with its surface in humps and depressions can have surface water penetrating to a depth of 46 cm (Cooper 1965 p. 21).

It is not possible to make, with the sampler, precise analysis of the vertical stratification of fauna in coarse materials in a stream; nevertheless, subdivision into three layers would be possible. Examination of the fauna at different localities, and at different depths in one locality, in relation both to particle sizes and to quantities of dead organic material, is therefore possible. It is unlikely that the abundance of animals can be accounted for solely in terms of one of these factors (see Ulfstrand 1967; Cummins and Lauff 1969; Eglishaw 1969).

It is not possible to compare the densities of animals found in the examples given with those obtained by other workers. The factor of size selection by other methods, apart from other differences, makes comparison impossible.

One generalization to emerge from the size-frequency distribution is that the times when most insects are present in streams are when most hatching is taking place. Thus, the claim that aquatic insect are scarce at certain times of the year (e.g., late autumn in temperate localities) is likely to be founded on size selection. It is possible that more insects occur then than at any other time of year, but they are present mainly as small instars.

Estimation of production of communities of stream invertebrates is very difficult as many species of different sizes with varied life histories, frequently living in a heterogeneous environment subject to instability, have to be dealt with. Its calculation is dependent, however, on repeated estimates of stocks. Comprehensive data of the complete size distribution of an invertebrate at various stages through its life cycle can be obtained with the sampler and permit direct calculation of rates of production for the population. An accurate measurement of invertebrate stock in riffles is also a requirement for calculating the theoretical capacity of riffles to produce invertebrate drift (see the treatment in Ulfstrand 1968), frequently the main food of the fishes.

The paucity of the hydrologist's methods of sampling bed material is apparent from texts on applied hydrology (e.g., Chow 1964), which mention only grabs and bucket-type samplers. One method, for coarse materials, that provides a representative sample of a whole reach of a stream, is to pick up 100 individual particles from the bed at random. Small sizes are not included, and the stream must be wadeable (Wolman 1954). The sampler described here would seem to offer the geomorphologist the advantage of being able to collect materials down to 50 μ and to select the depth down to which they should be taken. Sizes too large for the sampler can, of course, be recorded independently. The significance of size distributions of bed materials for geomorphologists is discussed by Leopold et al. (1964).

Finally it has been assumed that no biases occur in the sampling procedure of sufficient magnitude to invalidate the results obtained, i.e., that no significant amounts of material are lost from the sampler or gained by it from the outside. This assumption is based on close observation while samples were being taken. Nevertheless, it could be argued that in the absence of any experimental assessment of the efficiency of the sampler it is not possible to say anything about bias. Presumably any errors are most likely to be caused by losses; additions of silt or animals from outside would be small in relation to the total material collected. Losses are likely to be of small particle sizes and therefore of greatest consequence in the estimation of invertebrates.

Bias must be considered with reference to the purpose of the study; consistent bias might not be a serious disadvantage. Assessment of bias would require careful experimental studies in flumes with known contents of silt (e.g., the flume figured in Cooper 1964 p. 38). In view of this it should be borne in mind, when an investigation is being carried out in which great accuracy is necessary, that, even if the sampler gives results superior to those obtained by other methods, possible sources of bias have not been experimentally assessed.

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Effects of Feeding Level on Circulus Formation on Scales of Young Sockeye Salmon (*Oncorhynchus nerka*)

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Feeding level exerted a significant influence on scale growth of young sockeye salmon (*Oncorhynchus nerka*) maintained for 2 months at a temperature of 13.98 ± 0.29 C and a constant photoperiod of 12 hr light and dark. The number and spacing of circuli, scale radius, and length and weight of the fish were all correlated with the feeding level. Two checks at most were observed on scales of fish in the experimental populations. At midpoint of the experiment, incidence of a first check was highest among fish that had been held at a high feeding level. At the end of the experiment a second check was apparent only on scales of fish that had the feeding levels raised at midpoint of the experiment. There were significant correlations between the nucleus radius of the scale and length and weight of the fish.

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SCALES of Pacific salmon are used in both research and management to estimate age of the fish. Accuracy of age data is important. A typical salmon scale bears a series of circular corrugations (called circuli or sclerites) on its outer surface, confined almost entirely to the anterior part of the scale. Variation in growth of the fish is directly correlated with growth of the scale (width); however, formation of circuli does not necessarily change at the same rate (Clutter and Whitesel 1956). Thus, when growth of fish slows in winter, circuli tend to be closer together, forming bands of closely spaced circuli. Such bands are often referred to as "annual rings" in which the last narrowly spaced circulus of the annual ring preceding the first, more widely spaced circulus, is called the "annulus." The age of the fish is determined by counting these annuli. Other closely spaced bands of circuli, or "false annual rings," may occur at various times of the year as a result of other factors. In this paper the last narrowly spaced circulus of the false annual ring preceding the first, more widely spaced circulus, is defined as a "check." A false annual ring may consist of as few as two circuli. When both true and false annual rings are present on the scale, aging is difficult, and errors frequently occur (Godfrey et al. 1968).

A better understanding of the effects of various environmental factors on circulus formation can provide a better basis for aging salmon from their scale, providing increased accuracy. The purpose of this paper is to report the effects of three feeding levels on circulus formation on scales of young sockeye salmon (*Oncorhynchus nerka*), when light and temperature were held constant.

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Materials and Methods

EXPERIMENTAL STOCK

In the fall of 1968 approximately 5000 fertilized eggs from sockeye salmon from the lower Babine River, northern British Columbia, were transported by plane to the Nanaimo Station. These were reared in incubator stacks. When the yolk sacs had been absorbed, the fry were transferred to a 6-ft diam circular holding tank. Here they were treated according to standard fish culture practices and reared until they had formed scales, at an approximate length of 38 mm (Foerster 1929; Clutter and Whitesel 1956).

On June 20, 1969, 100 fry were selected without known bias from this stock of 5000 fry and their fork lengths measured in millimeters. The mean and standard deviation of their lengths were 47.65 ± 4.34 mm. On June 24, 425 fish whose lengths came within 1 SD of the mean were selected and groups of 25 were randomly assigned to 16 tanks. The remaining 25 fish were sampled for scales, measured for length and weight, and preserved in 10% formalin for examination.

SIZE OF EACH EXPERIMENTAL POPULATION

Only five fish would have been required for each experimental population, assuming that the variance between individuals within a population would be less

than one-third the variance between populations at different feeding levels (i.e., at the 95% confidence level, variance must be less than one-third between treatments for the differences to be significant). We started with 25 fish to allow for high mortalities.

Selection of Feeding Levels

Feeding was ad libitum twice a day, and total food for 1 day was defined as the "ration." The frequency at which the ration was offered was defined as the "feeding level." Three feeding levels were chosen: level 1 — one ration per day; level 2 — one ration every 4th day; and level 3 — one ration every 7th day. These feeding levels were based on experiments by Brett and Higgs (1970), which indicated that at 14 C, only 1% of the stomach contents of a juvenile sockeye salmon would be present 27 hr after feeding. At this temperature, fish at level 1 would always have food in their stomachs; those at level 2 would have empty stomachs for more than 70 hr; and those at level 3 would have empty stomachs for more than 5 days.

Experimental Apparatus

As the effect of feeding level only was to be examined, water temperature, water flow, and light period were held constant. To ensure freedom from noise and interference, experimental apparatus was isolated in a separate room, which was sealed to exclude outside light. Within the room, a series of eight circular fiberglass tanks was installed along two walls. Each tank held 10 gal of water at a mean temperature of 13.98 ± 0.29 C. Individual flow meters ensured equal flow to all tanks. The experimental area was illuminated by fluorescent lights automatically controlled to give 12 hr of light in each 24-hr period.

Experimental Design

The experiment was designed so that results could be statistically assessed by mixed model of analysis of variance (Table 1). The 15 experimental populations were designated by letters A–O, and population P provided length and weight data and scale characters at the beginning of the experiment, 2 weeks after the fish were introduced into the tanks on June 24. Population P was held at level 1 from June 24 to July 8, on both of which dates it was sampled to provide a starting point for body measurement and scale character.

Feeding levels of populations A–L (Table 1) were held constant throughout the period July 9–August 5. There were four replicates at each feeding level. On August 6 the feeding level of each of the groups was changed and maintained to September 9. There were two replicates at each combination of feeding levels. Populations M, N, and O were maintained at a specific feeding level throughout the experiment. Data from populations A–F were obtained twice, the first time on August 5 when fish were anesthetized with M.S. 222, measured, and returned to their tanks, and again on September 9. Data from populations G–O were obtained only on September 9.

Table 1. Experimental design, showing three feeding levels (numerals 1, 2, or 3), periods during which 15 populations were maintained at various levels, and sampling dates (S).

Population	July 9–Aug. 5		Aug. 6–Sept. 9	
A	1	S	2	S
G	1		2	S
D	1	S	3	S
J	1		3	S
B	2	S	3	S
H	2		3	S
E	2	S	1	S
K	2		1	S
C	3	S	1	S
I	3		1	S
F	3	S	2	S
L	3		2	S
M	1			S
N	2			S
O	3			S

Sampling and Scale Measurements

Each experimental population was placed in its respective tank on June 25 and all were fed an ad libitum diet daily until July 8; when population P was sampled on July 9 the other populations were placed on specific feeding levels. On August 5, populations A–F were measured, weighed, and scales removed from the part of the body just below the insertion of the dorsal fin and two or three rows above the lateral line (see International North Pacific Fisheries Commission 1957). Fish in all experimental populations were sampled in this manner on September 9.

In the laboratory, impressions of the scales were made in acetate plastic cards, using the method described by Clutter and Whitesel (1956). Scale impressions were then examined under a Leitz Prado-X, 123, microprojector equipped with plano lenses. When possible, three scales from each fish were examined to reduce the chances of error.

Images of the scale impressions were projected with a magnification of 254× onto sheets of 1-mm grid graph paper. Each scale impression was oriented so that the longest axis (determined by measurement) of the image lay along the lines of the grid, and the center of the nucleus was at the edge of the sheet. The outer edges of the images of each circulus from the nucleus to the edge of the scale were then marked on the graph paper, as were all checks.

For each scale, the following counts and measurements were made: N = radius of the nucleus (distance along longest axis of scale from center of nucleus (determined by eye) to outside edge of first circulus); A = radius of the scale (distance along longest axis of

scale from center of nucleus to edge of scale); A–N = radius of scale minus radius of nucleus; C = total number of circuli (counted along longest axis of scale from center of nucleus to edge of scale); A/C = average space between circuli; A–N/C–1 = average space between circuli when the radius of the nucleus and the first circulus is not included.

For each fish, values of measurements from three scales were averaged and tabulated.

Statistical Analyses of Data

Analysis of variance is simplified when the number of individuals in each population is the same. Therefore, the numbers in populations A–F were reduced by use of random number tables to 23 (the smallest population observed) for analyses of data to August 5. The numbers in populations A–L were further randomly reduced to 19, for the analyses of data on September 9.

Analysis of variance provided information on: (*i*) effect of feeding levels on scale formation and length and weight of fish; (*ii*) within feeding levels, effect of tanks on scale formation and length and weight of fish; (*iii*) interaction of tanks and feeding levels on scale formation and length and weight of fish.

Further statistical analyses produced correlation matrices between all combinations of variables within each feeding level. Means were compared in several instances with a Duncan's Multiple Range Test (Li 1964).

Results

Population P

The mean number of circuli for fish in population P on June 24 was 4.42 circuli (Table 2). A check was not apparent on any of the scales. By July 9, the mean number of circuli was 6.47, an average increase of 2.05 circuli over a period of 2 weeks of daily ad libitum diet. A check at an average of 3.69 circuli was observed on 13 of 24 scales (54%).

As all populations received identical treatment up to July 8, it was assumed that the mean number of circuli on scales of fish in population P was representative of the other experimental populations.

Populations A to F, for Period July 9–August 5

Comparison of mean lengths and various scale characters of populations A–F on August 5 with population P on July 9 (Table 3) indicated the following: (*i*) at level 1 (populations A and D), there was a significant increase in mean length, weight, number, and spacing of circuli and scale radius (A–N); (*ii*) at level 2 (populations B and E), each responded differently: for E there was a significant increase in mean length, weight, spacing of circuli, and scale radius (A–N), but no significant change in the mean number of circuli;

Table 2. Mean, standard deviation, and standard error of the various measurements of the 25 fish in population P on June 24 and July 9.

	June 24			July 9		
	$\bar{x}$	SD	SE±	$\bar{x}$	SD	SE±
Length (*mm*)	46.48	2.39	0.48	52.79	3.31	0.67
Wt (*g*)	0.77	0.11	0.02	1.61	0.34	0.07
Total circuli (C)	4.42	0.89	0.18	6.47	0.83	0.17
Scale radius (A–N)	14.82	4.66	0.93	20.13	4.11	0.84
Circuli spacing (A–N/C–1)	4.37	0.71	0.14	3.75	0.64	0.13
No. circuli to check	0	0	0	3.69	0.63	0.17

Table 3. Comparison of mean measurements of body and scale characters for populations A–F sampled on August 5 and population P sampled on July 9 using Duncan's Multiple Range Test. Means not underscored by a common line differ significantly at the 5% level.

Length	F 52.7	P 52.8	C 53.6	B 54.0	E 56.4	D 67.3	A 67.4
Wt	P 1.61	C 1.65	F 1.65	B 1.76	E 2.10	D 3.72	A 3.75
Total circuli (C)	F 5.27	C 5.50	B 5.65	E 6.42	P 6.47	D 9.36	A 9.69
Scale radius (A–N)	F 18.9	P 20.1	C 20.4	B 20.8	E 26.3	D 39.1	A 39.3
Circuli spacing (A–N/C–1)	P 3.75	B 4.38	F 4.51	A 4.54	C 4.60	D 4.69	E 5.03

for B there was a significant increase in the spacing of circuli, a significant decrease in the mean number of circuli. However, there was no significant change in mean length, weight, and scale radius (A–N); (*iii*) at level 3 (populations C and F), there was no significant change in mean length weight, and scale radius; both demonstrated a significant decrease in mean number of circuli and a significant increase in mean spacing of circuli.

Analysis of variance of data from tanks A to F indicated there were significant differences (at the 5% level) in the means of fish length ($F = 198.76$, degrees of freedom (df) 2, 132), weight ($F = 232.36$, df 2, 132), number of circuli ($F = 93.08$, df 2, 132), and scale radius (A–N) ($F = 105.78$, df 2, 132) among populations maintained at different feeding levels. There were no differences in the means of fish length ($F = 0.32$, df 1, 2)i weight ($F = 0.93$, df 1, 2), number of circul, ($F = 0.002$, df 1, 2), scale radius (A–N) ($F = 0.36$, df 1, 2), spacing between circuli (A–N/C–1) ($F = 1.15$, df 1, 2), and nucleus radius (N) ($F = 0.01$, df 1, 2) in populations maintained at the same feeding levels. For the scale radius (A–N) ($F = 3.52$, df 2, 132) and spacing of circuli (A–N/C–1) ($F = 3.53$, df 2, 132), there were significant interactions between feeding levels and tanks, demonstrating that the treatments did not have a consistent effect on each replicate population.

On many scales a check was observed on the average at the fourth circulus from the center of the scale nucleus (Fig. 1, 2; Table 4). The frequency of occurrence of the check on the scales of fish from different populations varied considerably (Table 4); 45 of 46 scales from fish maintained at level 1 had a check, whereas only 22 of 46 at level 2, and 11 of 46 at level 3, had checks.

As was expected, there were significant positive correlations (at the 5% level of significance) between length and weight of the fish and number of circuli and scale radius among fish maintained at the three feeding levels. Also, at levels 1 and 3 there were significant positive correlations of fish length, weight, and nucleus radius (for level 1, $r = 0.388$ and 0.451 respectively, and level 3, $r = 0.351$ and 0.445 respectively; $n = 46$). At level 2, only fish weight and nucleus radius were significantly and positively correlated ($r = 0.333$, $n = 46$). At levels 1, 2, and 3, weight of fish and space between circuli were significantly and positively correlated ($r = 0.296$, 0.388, and 0.445 respectively; $n = 46$). Only at level 2 was there a significant positive correlation between fish length and space between circuli ($r = 0.295$; $n = 46$).

Populations A to F, for Period August 5–September 9

Comparison of mean lengths and various scale characters on September 9 with those on August 5 (Table 5) indicated the following: (*i*) mean length of fish — a significant increment in length in populations C, D, E, F, with the greatest increment occurring when fish at levels 2 and 3 were changed to level 1 (populations C and E). There was no significant increment in size when fish at levels 1 and 2 were changed to levels 2 and 3 respectively (populations A and B); (*ii*) mean weight of fish — a significant increment in weight in populations C, E, F, when fish at levels 2 and 3 were raised to levels 1 and 2. There was a significant loss when fish at levels 1 and 2 were subjected to level 3 (populations B and D). There was no significant change in weight of fish at level 1 when they were subjected to level 2 (population A); (*iii*) mean total circuli — a significant increment in circuli in all populations with the greatest increment occurring when fish at levels 2 and 3 were raised to level 1 (populations C and E); (*iv*) mean scale radius (A–N) — the scale radius varied in the same manner as observed for the mean total number of circuli; (*v*) there was a significant decrease in the mean spacing of circuli (A–N/C–1) in populations E and F during the August 5 to September 9 period, but no significant changes occurred in the remaining populations.

A second check was observed on a number of scales at a mean value of 8.70 circuli from the center of the scale nucleus (Fig. 1, Table 4). The incidence of the second check on scales of fish from the different populations varied considerably. All scales of fish whose feeding level had been raised had a second check, whereas those whose feeding levels were reduced did not.

Populations A to L, for Period July 9–September 9

Comparison of mean lengths and various scale characters of populations G–L on September 9 with population P on July 9 (Table 6) indicated the following: (*i*) mean length of fish — a significant increment in length in all populations except B, which did not differ significantly from P. Population B was subjected to levels 2 and 3; (*ii*) mean weight of fish — a significant increment in weight in all populations except B, H, and F, which did not differ significantly from P. Populations B, H, and F were subjected to levels 2 and 3; (*iii*) mean total circuli — a significant increment in circuli in all populations except B, which did not differ significantly from P; (*iv*)

FIG. 1. Scale of a sockeye salmon from one of the experimental populations sampled on September 9, showing the two checks (arrows).

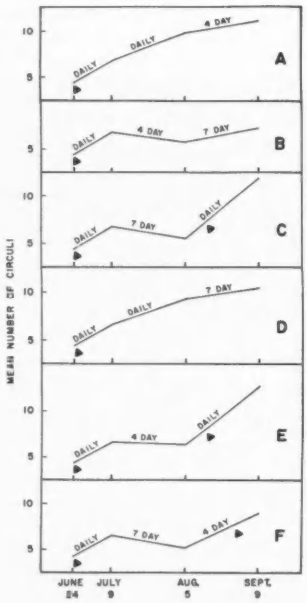

FIG. 2. Mean number of circuli on scales of sockeye of experimental population P on June 24 and July 9, and experimental populations A–F on August 5 and September 9. Arrows indicate the mean number of circuli to each check.

TABLE 4. Mean number of circuli to each check and in total, on scales of fish of each population on August 5 and September 9. The frequency of occurrence of each check in each population is also given.

Population	Feeding levels	No. fish	No. times 1st check present	Mean no. circuli to 1st check	Mean total circuli on Aug. 5	No. times 2nd check present	Mean no. circuli to 2nd check	Mean total circuli on Sept. 9
				August 5				
A	1	23	22	4.11	9.69			
B	2	23	7	4.47	5.65			
C	3	23	4	4.24	5.50			
D	1	23	23	4.63	9.36			
E	2	23	15	3.84	6.42			
F	3	23	7	3.88	5.27			
				September 9				
A	1 to 2	19	19	4.14	9.69	0		11.00
B	2 to 3	19	16	4.79	5.65	0		7.10
C	3 to 1	19	18	4.24	5.50	18	7.81	11.77
D	1 to 3	19	19	4.77	9.36	0		10.41
E	2 to 1	19	19	4.31	6.42	19	8.57	12.60
F	3 to 2	19	19	4.05	5.27	19	7.72	9.00
G	1 to 2	19	19	4.54		0		11.67
H	2 to 3	19	11	5.05		0		9.08
I	3 to 1	19	19	3.82		19	7.23	10.93
J	1 to 3	19	18	5.13		0		11.79
K	2 to 1	19	19	4.20		19	8.04	12.18
L	3 to 2	19	19	4.14		11	7.94	8.83
M	1	20	20	5.44		3	8.55	13.46
N	2	25	25	4.75		1		8.33
O	3	15	14	5.23		0		7.70

mean scale radius (A–N) — a significant increment in scale radius in all populations; (*v*) mean spacing of circuli (A–N/C–1) — a significant increase in circuli spacing in all populations except F and H, which did not differ significantly from P. Populations F and H were subjected to levels 2 and 3.

Analysis of variance indicated that between populations subjected to different combinations of feeding levels (1 to 2; 1 to 3; 2 to 3; 3 to 2; 3 to 1; and 2 to 1), there were significant differences in the means of fish length ($F = 107.75$, df 5, 216), weight ($F = 106.35$, df 5, 216), number of circuli ($F = 72.53$, df 5, 216), scale radius (A–N) ($F = 21.17$, df 5, 216), nucleus radius ($F = 3.16$, df 5, 216), and spacing between circuli (A–N/C–1) ($F = 21.17$, df 5, 216). There were no differences in the means of fish length ($F = 0.16$, df 1, 5), weight ($F = 5.16$, df 1, 5), number of circuli ($F = 0.92$, df 1, 5), scale radius (A–N), ($F = 2.59$, df 1, 5), nucleus radius ($F = 1.51$, df 1, 5), and spacing between circuli (A–N/C–1) ($F = 0.31$, df 1, 5) in populations maintained at the same combinations of feeding levels. However, for the number of circuli ($F = 8.05$, df 5, 216), scale radius (A–N) ($F = 2.79$, df 5, 216), nucleus radius ($F = 4.50$, df 5, 216), and spacing between circuli (A–N/C–1) ($F = 5.74$, df 5, 216), there were significant interactions between feeding levels, demonstrating that the treatments did not have a consistent effect on each replicate population.

If the data from all populations A–L are combined (Table 4), then the results indicate: (*i*) a check at a mean value of 4.42 circuli from the center of the scale nucleus on 215 of 228 scales (94%) from the 12 populations. The majority of fish in each population had this check on their scales. Fish of population H (level 2 to 3) differed from the remainder in that only 11 of the 19 fish had this check; (*ii*) in six populations (C, E, F, I, K, and L) a second check at a mean value of 7.88 circuli from the center of the scale nucleus. In every instance, these were populations where the feeding level had been raised. For the remaining six populations where the feeding level had been reduced, a second check was not observed.

Again, as was indicated during the July 9–August 5 period, there were significant positive correlations between length and weight of fish and number of circuli and scale radius among fish maintained at the various combinations of feeding

levels. Also, in four of the six feeding level combinations (groups AG, DJ, EK, FL), there were significant positive correlations between fish length, weight, and nucleus radius (the value of r varied from 0.328 to 0.636; n = 38). In two of the six feeding level combinations (groups CI, FL), there were significant positive correlations between fish length, weight, and space between circuli (the value of r varied from 0.326 to 0.536; n = 38). In two of the feeding level combinations (groups

TABLE 5. Comparison of mean measurements of body and scale characters for populations A–F sampled on September 9 with those sampled on August 5. (Populations reduced to 19 fish on September 9 for statistical purposes — see Materials and Methods.)

		Populations											
		A		B		C		D		E		F	
		Aug. 5	Sept. 9	Aug. 5	Sept. 9	Aug. 5	Sept. 9	Aug. 5	Sept. 9	Aug. 5	Sept. 9	Aug. 5	Sept. 9
Length (*mm*)	$\bar{x}$	67.4	69.7	54.0	54.8	53.6	70.8	67.3	69.7	56.4	74.0	52.7	59.6
	n	23	19	23	19	23	19	23	19	23	19	23	19
	t	1.402		0.588		17.829[a]		1.983[d]		15.591[a]		5.230[a]	
Wt (*g*)	$\bar{x}$	3.75	3.48	1.76	1.33	1.65	3.69	3.72	2.95	2.10	4.25	1.65	1.91
	n	23	19	23	19	23	19	23	19	23	19	23	19
	t	1.094		3.085[a]		13.221[a]		3.926[a]		11.882[a]		1.793[d]	
Total circuli (*C*)	$\bar{x}$	9.69	11.00	5.65	7.10	5.50	11.77	9.36	10.41	6.42	12.60	5.27	9.00
	n	23	19	23	19	23	19	23	19	23	19	23	19
	t	3.189[a]		3.067[a]		15.969[a]		3.216[a]		16.443[a]		9.553[a]	
Scale radius (A–N)	$\bar{x}$	39.3	47.9	20.8	26.7	20.4	49.8	39.1	44.1	26.3	51.4	18.9	31.1
	n	23	19	23	19	23	19	23	19	23	19	23	19
	t	3.884[a]		2.255[c]		13.965[a]		2.354[c]		12.571[a]		4.976[a]	
Circuli spacing (A–N/C–1)	$\bar{x}$	4.54	4.78	4.38	4.34	4.60	4.62	4.67	4.67	5.03	4.44	4.51	3.84
	n	23	19	23	19	23	19	23	19	23	19	23	19
	t	1.634		0.266		0.131		0.000		2.732[b]		3.759[a]	

[a]0.5% level of significance.
[b]1.0% level of significance.
[c]2.5% level of significance.
[d]5.0% level of significance.

TABLE 6. Comparison of mean measurements of body and scale characters for populations A–L sampled on September 9 and population P sampled on July 9, using Duncan's Multiple Range Test. Means not underscored by a common line differ significantly at the 5% level.

Length	P	B	H	L	F	I	A	D	J	C	G	K	E
	52.8	54.8	57.6	58.4	59.6	69.4	69.7	69.7	70.3	70.8	72.4	72.6	74.0
Wt	B	P	H	F	L	D	A	I	J	C	G	E	K
	1.33	1.61	1.77	1.91	2.15	2.95	3.48	3.50	3.63	3.69	3.74	4.25	4.42
Total circuli (C)	P	B	L	F	H	D	I	A	G	C	J	K	E
	6.47	7.10	8.83	9.00	9.08	10.41	10.93	11.00	11.67	11.77	11.79	12.18	12.60
Scale radius (A–N)	P	B	H	F	L	D	I	A	J	C	E	K	G
	20.1	26.7	30.4	31.1	33.3	44.1	45.1	47.9	49.2	49.8	51.4	54.0	55.1
Circuli spacing (A–N/C–1)	P	F	H	L	B	E	I	J	C	D	A	K	G
	3.75	3.84	3.84	4.26	4.34	4.44	4.53	4.56	4.62	4.67	4.78	4.81	5.18

AG, DJ), there were significant positive correlations between nucleus radius and number of circuli (r = 0.342 and 0.492; n = 38).

Populations M, N, O, for Period July 9–September 9

Comparison of mean lengths and weights and various scale characters on September 9 with population P on July 9 (Table 7) indicated the following: (*i*) fish on level 1 (population M) had a significant increase in mean length, weight, and number of circuli; (*ii*) fish on level 2 (population N) had a significant increase in mean length, weight, and number of circuli; (*iii*) fish on level 3 (population O) did not increase significantly in mean length, weight, or number of circuli; however, the mean scale radius increased significantly; (*iv*) there was a significant increase in the mean spacing of circuli in populations O and M over that of population P: that of population N did not change significantly. The spacing of circuli on scales of fish from populations M and O were not significantly different.

A comparison of the variables of fish in populations M–O indicated significant differences in mean length, weight, number, and spacing of circuli (Table 7). There was no difference in the mean nucleus radius between populations M, N, P, or M and O but there was between O and N, and O and P.

A check was observed at a mean value of 5.14 circuli on all but one of the scales (60 fish) from these populations (Table 6).

Table 7. Comparison of mean measurements of body and scale characters for populations M, N, and O sampled on September 9 and population P sampled on July 9, using Duncan's Multiple Range Test. Means not underscored by a common line differ significantly at the 5% level.

Length	O 52.7	P 52.8	N 57.0	M 78.5
Wt	O 1.57	P 1.61	N 2.15	M 5.94
Total circuli (C)	P 6.47	O 7.70	N 8.33	M 13.46
Scale radius (A–N)	P 20.1	O 30.1	N 30.4	M 58.9
Circuli spacing (A–N/C–1)	P 3.75	N 3.97	O 4.52	M 4.76
Nucleus radius (N)	P 17.57	N 18.23	M 18.76	O 20.51

A second check was observed only on 3 of the 20 scales from population M ($\bar{x}$ of 8.55 circuli), on 1 of the 25 scales from population N, but on none of the 15 scales from population O.

Discussion

The present experiment demonstrates that the feeding level had exerted a significant influence on the length and weight of fish, number of circuli, average spacing of circuli, scale radius, and formation of checks.

The observed differences in mean number of circuli and mean scale radius of fish maintained at different feeding levels were expected, as there were significant differences in length and weights of fish. Clutter and Whitesel (1956) demonstrated positive correlations between scale growth and length of fish.

Analysis of variance indicated the mean circuli spacing A–N/C–1 varied significantly between feeding levels on September 9. In fact, there was a significant correlation (r = 0.77, n = 12, $P < 0.01$) between mean circulus spacing of populations on September 9 and number of days each were fed ad libitum. This agrees with earlier work on effects of diet on the growth of trout scales (Gray and Setna 1931; Bhatia 1931; and Wingfield 1940). In the above studies the differences in circulus spacing were produced by extremes in diet; present studies indicate that the spacing of circuli is more sensitive to small changes in diet.

On August 5, when populations A–F were sampled, 56% (45 of 46 fish on level 1, 22 of 46 fish on level 2, and 11 of 46 fish on level 3) had formed a check at approximately the fourth circulus on their scales (Fig. 1). This check was most likely forming on July 9, as examination of scales removed at this time from fish of population P showed a check on 13 of 24 scales (54%), at a mean value of 3.69 circuli.

To speculate on the cause of this check it is necessary to examine a more detailed history of these fish before the beginning of the experiment. Up to June 24, they were part of a stock of 5000 fry in a single tank, where conditions for growth were not equal for all individuals (i.e., unequal distribution of food, possible pressure of hierarchies). On that date, the experimental populations were placed in the experimental tanks under improved conditions, i.e., small number of fish and equal diet. Possibly the increase in amount of food available to each fish from June 25 resulted in an increase in growth that was accompanied by formation of a check, prior to the beginning of

the experiment on July 9. When fish were sampled on August 5, 98% of those that had been continued on level 1 since June 24, 48% of those on level 2, and 24% of those on level 3, carried this check. However, when the same fish were sampled again on September 9, this check was apparent on all of level 1, 94% of those on level 2, and 98% of those on level 3. One explanation that would account for absence of the check on August 5 on many of the scales of fish held at the lower feeding levels is a delay in formation of the check.

When populations A–L were sampled on September 9, 94% (215 of 228) of the fish had a check at approximately the fourth circulus (Fig. 1). On 46% (105 of 228) of the scales a second had formed at approximately the eighth circulus (Fig. 1). This check was apparent only on scales of fish whose diets had been increased midway through the experiment. If populations A–F are considered (because they were sampled twice), it would appear from Fig. 2 that the second check formed from 8 to 21 days after the feeding level had been changed, assuming a constant rate of circulus formation. Thus there may be a time lag between exposure of a fish to an increase in diet and the subsequent record of this change on the scale (Major and Craddock 1962). Assuming there is always a delayed response of the scale to changes in a fish's environment, then it is possible that if the experiment had been allowed to continue a second check would eventually have been recognized on the scales of those fish whose diets had been decreased. Presence of a check on scales of fish whose diets had been increased, and absence of one on scales of those fish whose diets had been decreased, was not what had been expected in either instance.

There were significant correlations between the nucleus radius and length and weight of fish among many of the experimental populations sampled on August 5 and September 9.

To our knowledge, such growth correlations have not been previously reported. Bilton (1970) reported a positive correlation between size of female parent, size of egg, and size of resultant fry. As egg size influences growth of the fry, we believe it affects the size of the scale nucleus and the subsequent formation of circuli. Thus we speculate that there is a potential for growth that is correlated with egg size and is reflected in size of the scale nucleus and number and spacing of circuli when growth proceeds under optimum or near optimum conditions.

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Octopine in Postmortem Adductor Muscle of the Sea Scallop (*Placopecten magellanicus*)

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HILTZ, D. F., AND W. J. DYER. 1971. Octopine in postmortem adductor muscle of the sea scallop (*Placopecten magellanicus*). J. Fish. Res. Bd. Canada 28: 869–874.

Study of the rate and extent of octopine formation in ice-stored sea scallop meat (*Placopecten magellanicus*) confirms that it, rather than lactate, is a principal end product of carbohydrate metabolism in this species. Little or no octopine is detectable in fresh muscle dissected from live scallops, but on subsequent iced storage octopine accumulates, with reciprocal decrease in arginine, to maximum levels of about 1% in 6–10 days. An active octopine dehydrogenase, catalyzing the reductive condensation of arginine and pyruvate, was present, and lactic dehydrogenase activity was absent. Octopine accumulation occurs concomitantly with nucleotide degradation (dephosphorylation of adenosine 5′-triphosphate (ATP)). Its formation is linearly related to decline in muscle *p*H.

Octopine production had usually attained its maximum considerably before the point of inedibility was reached; hence, the use of octopine content as an index of quality in scallop meats seems limited to the early postmortem storage period, as confirmation of freshness.

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THE presence of octopine (N^{α}-(1-carboxyethyl)-arginine) in scallop muscle was first reported by Moore and Wilson (1937), who found about 1% in muscle iced 4–5 days. Subsequently, Irvin and Wilson (1939) observed that fresh muscle dissected from live scallops contained little or no octopine; rather, arginine accounted for some 90% of the total guanidine bases present. Within 3 days' storage of muscle slices at 0 C, however, octopine was found to increase markedly, and arginine to decrease reciprocally, suggesting that arginine was the precursor of octopine. It has since been shown (van Thoai and Robin 1959, 1961) that octopine is formed biosynthetically in the muscles of certain molluscs by reductive condensation of arginine and pyruvic acid, involving an $NADH_2$-dependent[1] enzyme, octopine dehydrogenase (ODH). The general reaction may be written

$$\text{arginine} + \text{pyruvic acid} + NADH_2 \xrightarrow{\text{ODH}} \text{octopine} + NAD^+ + H_2O.$$

Thus, the production of octopine in certain invertebrates is evidence for a major deviation from the classic glycolytic pathway whereby pyruvate is reduced to lactate, as in fish and mammalian muscle. Only very weak or no lactic dehydrogenase activity has been observed in those molluscs capable of synthesizing octopine (Robin and van Thoai 1961); these include mainly cephalopods, and a few lamellibranchs and gastropods (Regnouf and van Thoai 1970).

Little information was available on the rate and extent of octopine formation in scallop muscle post-mortem, especially during storage of the meats in ice for extended periods up to and beyond the limit of acceptability. The present study provides evidence of rapid accumulation of octopine to appreciable levels early in the postmortem period, of its apparent close association with other major postmortem biochemical changes, and of its limitations as a quality index in this species.

Materials and Methods

Procedures used in the handling of live scallops from the Georges Bank and inshore Nova Scotia fisheries, processing of adductor muscles for iced storage, composite sampling, and extraction of metabolic constituents with 6% perchloric acid were those described previously (Hiltz and Dyer 1970).

Octopine was determined with the α-naphthol: diacetyl reagent of Rosenberg et al. (1956). The standard curve (octopine from Sigma Chemical Co., St. Louis, Missouri) followed Beer's law over the range 5–80 μg

[1]Abbreviations used: AP, arginine phosphate; ATP adenosine 5′-triphosphate; AMP, adenosine 5′-monophosphate; ODH, octopine dehydrogenase; LDH, lactic dehydrogenase; $NADH_2$, NAD^+, reduced and oxidized forms of nicotinamide adenine dinucleotide (NAD), respectively; IP, inorganic phosphate.

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(0.02–0.3 μmole), provided all recommended precautions were observed in the procedure. It was found unnecessary to purify further the reagent-grade chemicals used.

Since the reagent is not specific for octopine, but reacts with mono- and di-substituted guanidines in general, octopine in neutralized perchloric acid extracts must first be separated from arginine by fractionation on a cation-exchange resin (AG 50W × 8, Calbiochem, Los Angeles, California; Na^+ form, *p*H 7), which retains arginine but not octopine (van Thoai and Robin 1959). We found that treatment of the neutralized extracts (diluted to contain up to 100 μg octopine per ml) by shaking (5 ml) for 15 min with 2 ml of a 1:2 slurry of the resin with water proved as efficacious in fractionating arginine and octopine as the use of small columns, and hence was adopted for routine use. After brief centrifugation to sediment the resin, a portion of the supernatant was used for octopine determination.

Thin-layer chromatography on precoated plates of cellulose MN 300 (Brinkmann Instruments, Rexdale, Ont.), developed in pyridine–*iso*–amyl alcohol–water (8:4:7 v/v) (Di Jeso 1968) and sprayed with Sakaguchi's reagent (Dawson et al. 1969), revealed that arginine and octopine were the only guanidine bases detectable in neutralized perchloric acid extracts of scallop muscle, and that octopine alone was present after shaking with resin. Thus, arginine may be determined as the difference between the α-naphthol:diacetyl reactives measured in the neutralized extract directly (arginine + octopine) and those measured after resin treatment (octopine). Arginine values obtained by difference in this manner agreed within 5% with those estimated according to van Thoai and Robin (1959), by elution of arginine from the resin with 0.1 N NaOH. Recoveries of arginine and octopine, and of mixtures of these, added to extracts before and after resin treatment, ranged from 99 to 104%.

AP was determined as the increase in IP after hydrolysis in 0.1 N HCl at 100 C for 1 min. IP, glycogen, ATP, AMP, and *p*H were determined as previously described (Hiltz and Dyer 1970).

Activity of octopine dehydrogenase was measured both in a crude extract (centrifuged homogenate of one part muscle and three parts water) and in a partially purified preparation. For the latter, the fraction of the crude extract precipitating between 30 and 70% of saturation with $(NH_4)_2SO_4$ was suspended in 5×10^{-2} M phosphate buffer *p*H 6, dialyzed for 24 hr against this buffer, and centrifuged at 200,000 *g* for 45 min. The muscle used for both preparations was quick-frozen within 30 min of shucking and stored at –26 C for 2 months. The assay mixtures contained arginine 4×10^{-2} M, sodium pyruvate 4×10^{-2} M, $NADH_2$ 2.5×10^{-4} M, and phosphate buffer 0.1 M at *p*H 6.6, the *p*H of optimum activity of ODH from *Pecten maximus* (van Thoai et al. 1969); assays were conducted at 30 C by following at 340 nm the oxidation of $NADH_2$ (Unicam SP 800 spectrophotometer, Pye Unicam Ltd., Cambridge, England). Protein nitrogen was determined with the biuret reagent (Snow 1950).

Results

Postmortem Changes in Guanidine Compounds during Iced Storage

Fresh muscle dissected from live scallops consistently contained high levels of AP and arginine, and little or no octopine. In a typical experiment (Fig. 1) the content of octopine increased rapidly on subsequent iced storage of the meats, from only traces at death to maximum levels of 35–40 μmoles/g (0.86–1.0%) in 6–8 days. Arginine levels increased early in the postmortem period due to the hydrolysis of AP, and then fell reciprocally as octopine accumulated. The formation of octopine was accompanied by a gradual decrease in muscle *p*H, ultimate levels of 6.1–6.2 being reached at the time of maximum octopine accumulation.

Although several other lots of scallop meats exhibited the same interrelations of phosphagen, arginine, and octopine shown in Fig. 1, considerable differences in the rate and extent of octopine accumulation were observed (Fig. 2). Variations were also noted in initial glycogen content and in the ultimate *p*H reached. Physiological condition or degree of antemortem activity or stress, or both, may have contributed significantly to the rate and extent of autolytic changes post-mortem. For example, scallops of lot A (Fig. 2), with a very slow rate of octopine formation, were thought to have spawned in captivity; this was supported by their very low glycogen content and by their capture

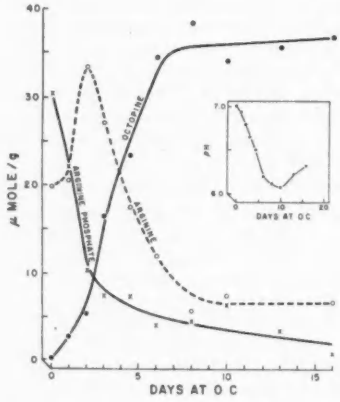

FIG. 1. Accumulation of octopine, and accompanying changes in arginine and arginine phosphate, in scallop adductor muscle stored in ice (lot E of Fig. 2). Each point represents a composite sampling of five different scallop meats. Insert: the accompanying changes in muscle *p*H.

in September, close to the spawning season for scallops of the Georges Bank fishery (Bourne 1964).

We found that quick-freezing of muscle samples at any given time post-mortem, or frozen storage at −26 C for at least 2 months, did not affect octopine contents. On the other hand, preliminary results indicate that significant increases in octopine content may occur on slow thawing (overnight at 4 C), especially in meats frozen prerigor.

Octopine Formation and Allied Nucleotide Degradative Changes

Nucleotide degradation occurred concomitantly with the postmortem accumulation of octopine during iced storage (Fig. 3). Although the rate of these reactions varied considerably among several lots of scallop meats examined (see Fig. 2), the relation of the changes to one another remained the same. The period of most rapid octopine production (e.g., within 2 days in Fig. 3) was always coincident with the dephosphorylation of ATP to AMP. A marked decline in *p*H accompanied these changes (see also Fig. 1); moreover, octopine production was found to be linearly related to *p*H fall (Fig. 4).

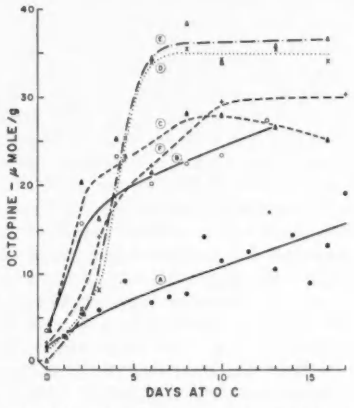

Fig. 2. Variation in rate and extent of octopine accumulation among six different lots of scallop meats during storage in ice.

Lot	Area caught	Month caught	Live-holding time[a] (*days*)	Initial glycogen content (%)	Ultimate *p*H reached
A	Georges Bank	Sept.	3 months	0.2	6.85
B	" "	Apr.	13	0.9	6.7
C	" "	June	1–2	2.9	6.2–6.4
D	Inshore, Lunenburg	Nov.	4 months	2.0	6.1
E	Bay of Fundy	May	4	1.6	6.1
F	Bay of Fundy	July	3	1.8	6.0

[a]Time held alive, without feeding, in laboratory aquaria with running sea water at 6–10 C before shucking and storage in ice.

Activity of Octopine Dehydrogenase

Activity of ODH in the crude and partially purified enzyme preparations was, respectively, 2.3 and 12.1 μmoles $NADH_2$ oxidized per min/mg protein (protein N × 6.25), under the assay conditions used. The partially purified preparation was stable for at least a week at 4 C. Activities,

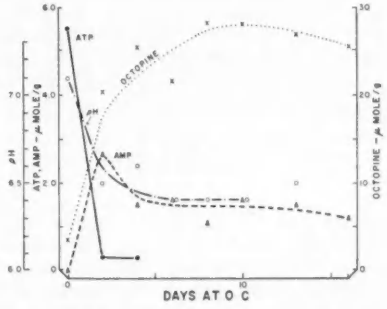

Fig. 3. Postmortem concurrence of octopine formation, *p*H fall, and ATP dephosphorylation in scallop muscle stored in ice (lot C, Fig. 2).

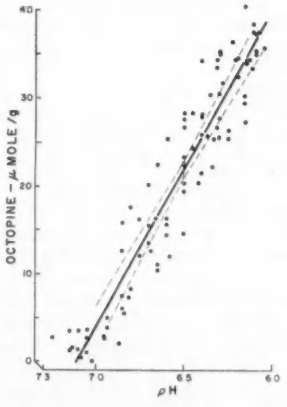

Fig. 4. Relation between octopine content and muscle *p*H in scallop adductor muscle during postmortem storage of various lots (11) in ice. *p*H values down to and including ultimate levels only are included. Y = 253–35.6X (n = 80), where Y is octopine content (μmoles/g) and X is *p*H. The 95% confidence limits are shown.

determined at three or more concentrations, were proportional to the amounts of enzyme preparation used. The activity of our partially purified preparation was similar to that reported for muscle of the European scallop, *Pecten maximus* (van Thoai et al. 1969).

In contrast, we could not demonstrate lactic dehydrogenase activity in the partially purified enzyme preparation, assayed in the presence of pyruvate and $NADH_2$ according to Kornberg (1955). In the crude extract, an "apparent" LDH activity of 0.03 units per mg was attributed to weak ODH activity. The required substrate, arginine, would certainly be present in water extracts of prerigor frozen scallop muscle; also, we found octopine to be formed as a product of the reaction, in stoichiometric relation to the $NADH_2$ oxidized. No lactate could be detected (levels <0.1 μmole/g) in the reaction mixtures, nor in neutralized perchloric acid extracts of muscle iced from 0 to 16 days.

Discussion

Formation of octopine from arginine and pyruvate is clearly one of the major biochemical processes occurring in scallop muscle postmortem; its accumulation begins from the moment of death, and, in general, increases rapidly to maximum levels of 25–35 μmoles/g (0.6–0.9%) after 6–10 days storage of the meats in ice (Fig. 2). These results are in accord with the early work of Moore and Wilson (1937), whose report of 1% octopine in muscles aged 4–5 days was the only previous record of this guanidine in *P. magellanicus*. The production of octopine appears to be stoichiometrically related to the loss of arginine; for example, in Fig. 1, the total of AP, arginine, and octopine is 50 μmoles/g initially, and 49, 49, 50, 48, and 48 μmoles/g at 2, 4.5, 6, 8, and 10 days respectively. Physiological condition, e.g., spawning, may affect the rates markedly. In lot A (Fig. 2) octopine formation was very slow, and arginine levels remained in the range 17–23 μmoles/g. Unfortunately AP was not determined. However, in a later experiment using scallops sampled post-spawning (October 1970), hydrolysis of AP was also very slow, only 40% in 7 days. The initial AP level was low, 9.5 μmoles/g, as was the total of AP, arginine, and octopine, about 30 μmoles/g. The slow rate of octopine formation in this lot may be due partly to the slow hydrolysis of AP, but the maintenance of high muscle *p*H levels may possibly have slowed the activity of ODH and the other enzymes of the glycolytic pathway.

Octopine appears to be a principal end product of carbohydrate metabolism in scallop adductor muscle. Its formation is one means whereby NAD^+ may be regenerated by oxidation of the $NADH_2$ produced at the triosephosphate dehydrogenase step in the pathway. In this respect, the $NADH_2$-dependent enzyme, ODH, replaces the role played by LDH in the metabolism of pyruvate in fish and mammalian muscle. This is supported by our finding that an active ODH was present in *P. magellanicus* muscle, and that LDH was absent. The "mutual exclusiveness" of these two dehydrogenases in a few other invertebrate species, including *P. maximus*, has been reported by Robin and van Thoai (1961). On the other hand, in some lamellibranchs, notably a clam, *Rangia cuneata*, a major product of glycogen degradation has been shown to be succinate, produced mainly by carboxylation of phosphoenolpyruvate (PEP) by a very active PEP carboxykinase (Stokes and Awapara 1968); these authors found that the disposition of pyruvate in this invertebrate may also proceed by way of transamination to alanine. It is not known as yet to what extent, if at all, these or other pathways are operative in scallop muscle. That glycogen is metabolized in some way other than by octopine formation in this species is indicated, since in those lots of scallop meats where glycogen breakdown occurred post-mortem (at least 50% metabolized by the end of the storage period), octopine production accounted for only 20–25% of the glycogen degraded. In other lots, there was little apparent glycogen breakdown post-mortem; however, the variation in glycogen content measured over the storage period was such that a glycogen decrease sufficient to account for the octopine formed would not have been detected (e.g., glycogen contents in lot E, Fig. 2, ranged from 1.4 to 1.9% over 10 days iced storage, with an average value ± SE of 1.6 ± 0.04 for eight determinations).

The observation that glycolysis and resultant octopine formation occurred concomitantly with ATP dephosphorylation in scallop muscle is a manifestation of the general phenomenon of the relation between glycolysis and nucleotide degradation, already well documented for mammalian and fish muscle (e.g., Bendall 1960; Fraser et al. 1967). Indeed, the interrelations of the changes in phosphagen (AP), ATP, *p*H, and octopine in scallop muscle are similar to those in phosphagen (creatine phosphate), ATP, *p*H, and lactate in vertebrate muscle, although the rates of these changes are much slower in scallop. In scallop, as in vertebrates, hydrolysis of phosphagen is the first major chemical change post-mortem (see Fig. 1), preceding decay of ATP, decline in *p*H and accumulation of glycolytic end products, the latter three processes occurring more or less simultaneously. The time course of octopine formation

in scallop may be represented by a *p*H-time curve, since, within the limits of *p*H 7.2–6.0, its production is linearly related to *p*H fall (Fig. 4). For 11 different lots of scallop meats examined, the relation $\frac{\Delta \text{ octopine}}{\Delta\ p\text{H}}$ averaged (± SE) 37 ± 0.9 μmoles/*p*H per g of muscle. In rabbit psoas muscle (Bendall 1960), and in cod (Fraser et al. 1967), the corresponding relation $\frac{\Delta \text{ lactate}}{\Delta\ p\text{H}}$ was 60–65 μmoles/*p*H per g, within the same *p*H range. Although this difference may possibly indicate a lower buffering capacity in scallop muscle (less acid production required to effect the same *p*H drop), the probability exists that acid is also produced in this species via some pathway(s) of glycogen degradation other than that leading to octopine formation (see above), e.g., via the Krebs cycle (succinate), or accumulation of other acidic intermediates. In this connection, it is noteworthy that the contribution to acidity by breakdown of AP and of ATP is apparently either negligible or not in excess of the buffering power of the muscle, since cooking of freshly dissected muscle resulted in no significant *p*H change (or glycogen breakdown), although complete hydrolysis of AP and dephosphorylation of ATP occurred.

Due to the relatively rapid rate of octopine production in iced scallop muscle, appreciable amounts of this guanidine accumulated within the confines of edibility. To test the value of octopine content as a quality index in scallop meats, taste scores, assigned by an experienced taste panel, were plotted against octopine contents; the regression line (Fig. 5) showed a highly significant ($p < 0.001$) linear relation, but indicated that maximum octopine production is reached at taste scores of 50–60, considerably in advance of the limit of acceptability (score of 40). Hence, octopine content as a quality index in scallop meats seems limited to the early postmortem storage period as confirmation of freshness. Hypoxanthine content, on the other hand, has shown greater potential as a quality index in this species (Hiltz and Dyer 1970).

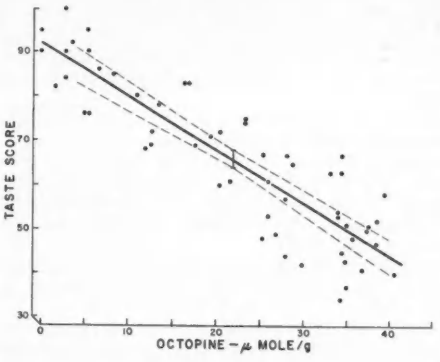

FIG. 5. Regression line of taste scores on octopine contents in several lots of scallop meats stored in ice. 95% confidence limits are shown; Y = 92–1.2X (n = 55), where Y is taste score and X is octopine content (μmoles/g). The meats were cooked by steaming, and evaluated by an experienced taste panel according to established procedures (Power et al. 1964).

Acknowledgments

We thank Messrs F. C. Read, National Sea Products, Lunenburg, N.S., and C. G. Murphy, Department of Fisheries and Forestry, Digby, N.S., for their cooperation in obtaining live scallops, and Mr D. E. Turner for excellent care and maintenance of the aquaria.

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NOTE ADDED IN PROOF

Since this work was accepted for publication, O'Doherty and Feltham (1971)[1] have reported the absence of ODH and the presence of LDH in extracts of adductor muscle of *P. magellanicus*. These results are at variance with the present work, in which the apparent low LDH activity in crude extracts was attributed, in fact, to ODH activity, and in which an active ODH was demonstrated. This latter finding is in agreement with the large amounts of octopine formed in scallop muscle post-mortem.

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[1]O'DOHERTY, P. J. A., AND L. A. W. FELTHAM. 1971. Glycolysis and gluconeogenesis in the giant scallop, *Placopecten magellanicus* (Gmelin). Comp. Biochem. Physiol. 38B: 543–551.

Egtved Virus: Demonstration of Neutralizing Antibodies in Serum from Artificially Infected Rainbow Trout (*Salmo gairdneri*)

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VESTERGÅRD JØRGENSEN, P. E. 1971. Egtved virus: demonstration of neutralizing antibodies in serum from artificially infected rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Canada 28: 875–877.

Rainbow trout (*Salmo gairdneri*) kept in a tank with tap water supply for 18 months at a water temperature of 8–13 C were given monthly intraperitoneal injections of nonpathogenic cell-culture-derived Egtved virus. After 1 year, a low titer of neutralizing antibodies to Egtved virus could be demonstrated in the trout serum. Such antibodies could not be demonstrated by the method applied in sera from rainbow trout exposed to natural infection with Egtved virus at two Danish trout farms.

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THE immune response of rainbow trout (*Salmo gairdneri*) to infectious pancreatic necrosis (IPN) virus was examined by Wolf and Quimby in 1969. It was demonstrated that rainbow trout injected with IPN virus produced high titers of neutralizing antibodies against IPN virus and that such antibodies were also produced by rainbow trout naturally infected by the virus.

Information about the immune response of rainbow trout to another trout-pathogenic virus, Egtved virus, the causative agent of viral hemorrhagic septicemia (VHS) (Jensen 1963, 1965), seems to be completely lacking in the literature about this disease.

The purpose of the present investigation was to determine whether neutralizing antibodies to Egtved virus could be produced in rainbow trout, and whether demonstration of such antibodies would be useful in diagnostic work.

Materials and Methods

FISH

For the immunization experiment, rainbow trout of about 150 g were provided from a trout farm where symptoms of VHS had never been observed. Virological examination of liver, kidney, and spleen from five fish was carried out before the start of the experiment, without isolation of Egtved virus.

Five fish were placed in a tank with a tap water supply at 8–13 C and were fed a pellet diet. Prior to experimental infection, the fish were anesthetized, individually tagged, and their blood was sampled by heart puncture. Once a month during a period of 9–18 months, three of the fish were given an intraperitoneal injection of 1 ml of Egtved virus, preceded by withdrawal of a blood sample from each of the fish. Two fish receiving no virus injections served as negative controls.

Sera from 35 rainbow trout, 4–8 years old and exposed through years to natural Egtved virus infection, were obtained from two trout farms where VHS had caused severe losses every winter and spring for 5–10 years. At the time of bleeding no symptoms of VHS has been observed among the fish for 6–8 months.

Negative-control sera were obtained from five fish at a trout farm with no history of VHS.

All the sera were virologically examined before use in neutralization tests.

RABBITS

To obtain positive-control sera, four rabbits were immunized with Egtved virus according to methods previously described (Vestergård Jørgensen 1968).

VIRUS

Egtved virus used in the experiment was fluid from RTG-2 cell cultures (Wolf and Quimby 1962) infected with Egtved virus strain F-1 (Jensen 1965) passages 80–95 in RTG-2 cells. Virus multiplication took place at 15 C and virus was harvested when maximal cytopathic effect (CPE) was observed 4–6 days after inoculation. The fluid was filtered through a 0.45-μ membrane (Millipore, HA) or centrifuged at 1000 g at 2 C, and usually contained $10^{6.0}$–$10^{6.5}$ $TCID_{50}$ per ml. Virus for inoculation into rabbits was of the same origin but concentrated by centrifugation at 30,000 g at 2 C. The concentration process usually led to a 50-fold increase of the infectivity titer.

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IPN virus used in demonstrating the specificity of the anti-Egtved sera was fluid, filtered as above, from RTG-2 cell cultures infected with IPN virus strain Sp, passages 20–25, isolated by the author at a Danish trout farm in 1968.

Cell Cultures

Monolayers of RTG-2 cells were grown in static tubes and bottles at 20 C in Eagle's Minimal Essential Medium with 10% fetal bovine serum. The medium was supplemented with 100 I.U. penicillin and 100 μg of streptomycin per milliliter. After inoculation, cell cultures were incubated at 15 C for 7 days after which they were examined for CPE.

To prevent loss of virus infectivity, growth medium was, unless otherwise stated, used throughout the experiment for dilution of serum and virus.

Virus Isolation Tests

Tissue for virological examination was homogenized in a mortar with quartz sand, and in a dilution of 10^{-1} –10^{-2} in PBS at *p*H 7.4 filtered as described for cell culture fluid. Of the filtrate, 0.1 ml was inoculated into each of two cell-culture tubes. Trout serum was diluted 10^{-1}, after which filtration and inoculation were carried out as above.

Serum-Neutralization Test

Serial twofold dilutions of unheated serum were mixed with a constant amount of Egtved virus, i.e., 100, 50, or 5 $TCID_{50}$ per 0.1 ml, and the mixtures were incubated at 15 C for 2 hr or at 4 C for 24 hr. After that, 0.2 ml of each mixture was inoculated into three replicate tube cultures. Control titrations of the actual virus amounts were carried out simultaneously by inoculating 5–10 tube cultures from each virus dilution. The infectivity titers were calculated by the Kärber method, whereas 50% end-point titers of the sera were calculated by the Reed–Muench method.

Plaque-Neutralization Test

Serial twofold dilutions of serum were mixed with a constant amount of 50–100 PFU of Egtved virus per 0.2 ml and reacted as above. Of each serum–virus mixture, 0.4 ml was then adsorbed for 1 hr at 15 C to a 32 cm^2 cell layer, which was subsequently overlaid with an 0.5% nutrient agar (Reinagar, Behringwerke) buffered with Tris to a *p*H of 7.6 (Kinkelin and Scherrer 1970). On the 5th day, 1 ml of a 1:1000 solution of neutral red in 0.9% NaCl was added to each bottle, and plaque count was made 4 hr later. Two bottle cultures were used per serum–virus mixture.

The titer of a serum in this test is expressed as the highest dilution giving a 50% reduction of the number of plaques counted in cultures with normal or pre-immunization serum (Casals 1967).

Results

By the serum-neutralization test, antibody titers varying from 1:231 to 1:4246 against 32 $TCID_{50}$ of Egtved virus were demonstrated in four rabbit sera. The neutralizing effect of the sera was specific, since it could be shown that 1:10 serum dilutions were unable to neutralize 32 $TCID_{50}$ of IPN virus per 0.1 ml (Table 1).

By the same technique, titers of 1:16 and 1:64 against 50 $TCID_{50}$ of Egtved virus could be demonstrated in one of the fish from the immunization experiment after 12 and 18 months, respectively.

The serum with a titer of 1:64 in the above test was additionally examined in a plaque-neutralization test and was repeatedly shown to have a titer of about 1:160 in that test when measured against 100 PFU of Egtved virus. The other two of the immunized fish had not developed detectable levels of antibody when they died in the 9th month of the experiment.

Table 1. Fifty percent end point titers of neutralizing antibodies to Egtved virus.

	Test dose of virus		
	5 $TCID_{50}$	32–50 $TCID_{50}$	100 $TCID_{50}$
20 trout sera; trout farm 1; VHS+	<1:10	<1:10	<1:10
15 " " " " 2; VHS+	<1:10	<1:10	<1:10
5 " " " " 3; VHS–	<1:10	<1:10	<1:10
Exptl. fish 1	–	1:64[a]	–
2	<1:10	–	–
3	<1:10	–	–
Positive control serum (rabbit)	–	1:4246	–

[a]1:160 in plaque-neutralization test with 100 PFU.

By the serum-neutralization test none of the 35 brood stock sera from VHS-attacked trout farms could be shown to contain neutralizing antibodies against Egtved virus, serum dilutions 1:10 being in all instances unable to neutralize 100, 50, or 5 $TCID_{50}$ per 0.1 ml. The same results were obtained whether serum-virus mixtures were reacted for 2 hr at 15 C or for 24 hr at 4 C.

Apparently viremia was not present in any of the fish at the time of bleeding, since virus was not isolated from any of the sera.

Discussion

The facilities of the laboratory were too modest to allow larger numbers of fish to be included in the immunization experiment for a long term. In spite of this, however, it is possible to draw one important conclusion, namely, that rainbow trout are able to produce significant levels of neutralizing antibodies to Egtved virus.

The late occurrence of antibody in the fish may be due to several factors, among which the relatively low water temperature may be most important (Sigel 1967). This factor, however, could not for technical reasons be regulated in the experiment.

Jensen (1965) reported that cell culture passage no. 30 of Egtved virus strain F-1 was unable to produce VHS in rainbow trout. This is in agreement with the fact that none of the above fish inoculated with passages 80–95 of F-1 showed symptoms of VHS during the experiment.

The absence of neutralizing antibodies in brood stock sera from VHS-attacked trout farms seems to indicate that demonstration of such antibodies in trout will hardly be of any practical value in determining whether a brood stock has been exposed to Egtved virus. This is true at least when the examination is carried out during the winter time, as was the case with this examination. The brood stock sera were examined only by the serum-neutralization test, since sufficient serum was not left for the plaque-neutralization test. Very small amounts of virus, however (5 $TCID_{50}$ per 0.1 ml), were used in the test and for that reason it seems unlikely that antibody could have been demonstrated by the plaque-neutralization test. The latter was found to be more sensitive than a serum-neutralization test with a virus dose of 50 $TCID_{50}$ per 0.1 ml.

Acknowledgments

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Enolase from Lobster (*Homarus americanus*)

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Enolase has been isolated from lobster muscle by acetone fractionation, heat treatment, ammonium sulfate fractionation, gel filtration, and ion-exchange chromatography. Preliminary characterization of the pure enzyme shows that the catalytic properties are very similar to those of the enolases from rabbit and fish.

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COMPARATIVE studies of functionally analogous enzymes of different phylogenetic origin represent an experimental approach to the elucidation of two different types of questions. Firstly, the results of such studies provide an independent basis for ordering organisms according to phylogenetic relations and can be used to establish biochemical evolutionary trees. These types of conclusions derived from comparative studies are well illustrated by the work on cytochrome C (Margoliash and Schejter 1966). Secondly, if one assumes that the "active site" of an enzyme was selected early and remained essentially unchanged during evolution, whereas other parts of the molecule adapted to the unique environments of individual species, a detailed comparison of the structure of analogous enzymes might reveal common invariant structural components of all the analogous enzymes. Such findings should provide an independent approach to the elucidation of the active site of enzymes. The success of this approach is well illustrated by the comparative work on the primary "active site" sequences of serine esterases (Hartley 1964).

The most useful enzymes for this kind of study must be those that are common to all species, and since glycolysis is a virtually ubiquitous biochemical process, glycolytic enzymes should be well suited for comparative studies. We have consequently undertaken to study enolases from a variety of organisms and have previously isolated and characterized enolase from rabbit muscle (Holt and Wold 1961; Winstead and Wold 1965) and from three species of Salmonidae (Cory and Wold 1966; Ruth et al. 1970) and compared their properties with those of the yeast enzyme (Malmström 1961). An enolase from the phylum Arthropoda was also included, lobster being selected as a suitable representative. This paper reports the isolation and preliminary characterization of enolase from lobster (*Homarus americanus*).

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Experimental

METHODS

Enolase activity was determined by the direct spectrophotometric method of Warburg and Christian (1942) according to standard procedures (Winstead and Wold 1966) with a Zeiss or a Cary 15 spectrophotometer thermostatted at 25 C. The standard assay medium contained 10^{-3} M D-glyceric acid 2-phosphate (tricyclohexyl–ammonium salt), 10^{-2} M $MgSO_4$, 0.2 M KCl in 0.05 M Imidazole buffer, *p*H 7.0. Since the lobster muscle extracts had high 280–260 mμ absorbance ratios, protein determination during the isolation was based on the absorption at 280 mμ, assuming an absorbancy of one for a 1 mg/ml solution of protein. Acrylamide disc-gel electrophoresis was carried out with the Canalco model 12 apparatus according to the procedure of Ornstein (1964) and Davis (1964). All reagents were of the highest available purity, and a commercial source of deionized, glass-distilled water was used throughout.

PREPARATION OF ENZYME

Unless other conditions are specified, all steps were conducted at 2–4 C. All centrifugations were carried out with the Sorvall Model 2B refrigerated centrifuge with the large angle head, maintained at 0–1 C. All samples were uniformly centrifuged at 9000 × *g* for 30 min. All precipitates were dissolved in a magnesium–imidazole buffer (MI-buffer) containing 0.05 M imidazole, 10^{-3} M $MgSO_4$, adjusted to *p*H 7.0 with HCl.

Step 1. Extraction

Live lobsters were decapitated and the muscle was quickly removed from the tail and the larger claws and frozen in approximately 100-g batches. The frozen tissue was stored at –20 C, and the enolase activity

appeared stable for several months under these conditions. After a year, however, some loss of activity was observed. About 100 g of tissue was blended with 2.3 volumes of cold versene solution (0.05% w/v of disodium dihydrogen ethylenediaminetetracetate dihydrate) in a Waring Blendor for 2 min, and then centrifuged.

Step 2. Acetone fractionation

The acetone was precooled to –20 C. Cold acetone was added rapidly to the muscle extract with efficient stirring, to give a 35% acetone concentration (54 ml acetone per 100 ml of extract, assuming the volumes to be additive), and the solution was centrifuged. The precipitate was discarded and the supernatant was brought to 50% acetone concentration by the further addition of 46 ml of acetone per 100 ml of the original muscle extract. The precipitate was collected and dissolved in MI-buffer to give a total volume about one-third of the original extract.

Step 3. Heat treatment

Sufficient amounts of $MgSO_4 \cdot 7H_2O$ were added to the above solution to give a 5% solution and the solution was brought to 50 C by swirling in a water bath maintained at about 70 C. After 2 min at exactly 50 C, the enzyme solution was cooled as rapidly as possible in an ice bath and centrifuged. The heat step is the least reproducible step in the procedure. In some instances, the yield was so low that no purification was achieved. By controlling both the temperature and the exposure time very carefully, however, the step generally gave sufficient enzyme enrichment to be deemed useful.

Step 4. Concentration by amnonium sulfate precipitation

Ammonium sulfate was added to the supernatant from the heat step to 40% saturation (22.8 g/100 ml supernatant), and after 15 min of continuous stirring, the resulting precipitate was removed by centrifugation and discarded. Additional ammonium sulfate was added to give 80% saturation (26 g/100 ml of heat supernatant), the solution was again stirred for ⅘ min, and the precipitate was collected by centrifugation. The precipitate was dissolved in IM-buffer to give a total volume of about 10% of the original muscle extract. Little or no purification was achieved in this step, which serves primarily as a concentration step prior to gel filtration.

Step 5. Gel filtration on Sephadex G-100

The concentrated fraction from the ammonium sulfate step was further purified by gel filtration. This step gave quite good recovery of the total enzyme activity, and it was found convenient to pass the sample through a relatively small column twice for optimal yield and purity. The only troublesome part of this step was the concentration step between the two gel filtration runs. Lobster enolase is completely inactivated by freezing, and lyophilization, therefore, could not be used. Preevaporation in the cold room gave good yields, but was time consuming. The method of choice was to pool the active fractions from the first run (the volume was usually four times larger than the starting volume), transfer the solution to a dialysis bag, and pack the dialysis bag in Aquacide. For the next 3–4 hr, fresh dry Aquacide was added to replace the wet material, and in this length of time the volume had been reduced by about 75%. This concentrated protein solution was then subjected to the second gel filtration run. The experimental details are given in the legend to Fig. 1, which also shows the elution pattern from the gel filtration steps.

Step 6. Chromatography on DEAE-cellulose

The high specific activity fractions from the second gel filtration step were pooled, and after adjusting the *p*H to 8.3, the sample was applied to a DEAE-cellulose column equilibrated with 10^{-2} M Tris·HCl buffer, *p*H 8.3. The column was eluted with 0.1 M KCl in the same Tris buffer, and then with a linear gradient from 0.1 to 0.4 M KCl. Experimental details are given in the legend to Fig. 2 in which a typical elution profile is illustrated. The high (constant) specific activity fractions from the DEAE column were pooled and used for the enzyme characterization. The enzyme was stable for a few days at 4 C without further treatment. For prolonged storage, a suspension in 75% saturated ammonium sulfate was found satisfactory.

Results

The purification of lobster enolase is summarized in Table 1. Attempts to crystallize the enzyme or to purify the DEAE-chromatography fraction further were unsuccessful. The DEAE fraction appeared pure by acrylamide disc-gel electrophoresis (Fig. 3) and also by the criterion of constant specific activity. The molecular weight of the enzyme was estimated by gel filtration on Sephadex G-100, using several known proteins as standards. From the elution position relative to the standards (Fig. 4) a molecular weight of 87,000 ± 3000 was obtained.

The catalytic properties of lobster enolase were tested according to published procedures (Cory and Wold 1966; Ruth et al. 1970) and gave results (Table 2) essentially identical to those obtained for mammalian (Holt and Wold 1961) and fish (Cory and Wold 1966; Ruth et al. 1970) enolases. Thus, the enzyme has an absolute requirement for divalent metal ions. Magnesium is the most efficient and gives maximum activation in the activity assay at a concentration of 10^{-2} M. The *p*H optimum, the Km, and Vmax are also very similar to those of enolases from other higher species.

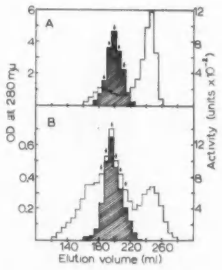

FIG. 1. Gel filtration on a 2.5 × 90-cm column of Sephadex G-100 equilibrated at 4 C with 0.05 M Imidazole buffer containing 10^{-3} M $MgSO_4$, *p*H 7.0 (MI-buffer). The column was eluted in the cold with MI-buffer at a flow rate of 30 ml/hr, and 6-ml fractions were collected. The crosshatched areas represent activity. (A) Gel filtration of 13 ml of the ammonium sulfate fraction containing 9300 units; (B) the fractions marked with arrows in (A) (36 ml) were pooled, concentrated to 10 ml by treatment with aquacide, and reapplied to the same column. Fractions marked with arrows were collected for DEAE chromatography.

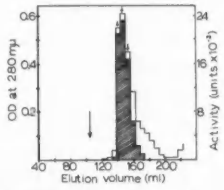

FIG. 2. Ion-exchange chromatography of the Sephadex G-100 fraction on a 2.5 × 60-cm column of DEAE-cellulose (DE-32, microgranular Reeve-Angel). The column was equilibrated with 10^{-3} M Tris HCl buffer, *p*H 8.3. The sample *p*H was adjusted to 8.3 and washed onto the column with 50 ml of the equilibration buffer. The column was next eluted with 50 ml of 0.1 M KCl in equilibration buffer and then with a linear gradient from 0.1 to 0.4 M KCl in equilibration buffer. The large arrow indicates the start of the gradient. The enolase peak eluted at about 0.12 M KCl. The flow rate was 1 ml/min and 6-ml fractions were collected. The crosshatched area represents activity. The fractions marked with arrows had constant specific activity, and were pooled as pure enolase.

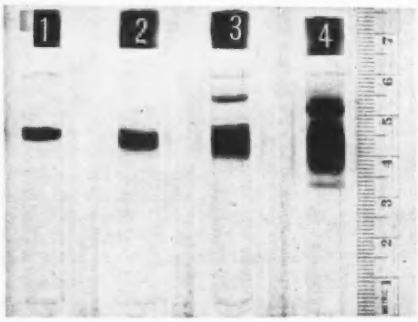

FIG. 3. Acrylamide patterns of lobsters enolase at different levels of purity; (1 and 2) pooled enolase fractions after DEAE chromatography (tube 1 contained 50 μg and tube 2, 150 μg); (3) enolase obtained from DEAE chromatography preceded by only one gel filtration step; (4) the enolase fraction after the ammonium sulfate step. The gels were run at *p*H 4.3 under standard conditions.

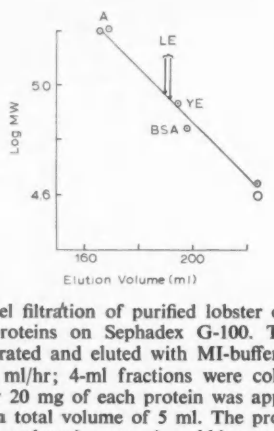

FIG. 4. Gel filtration of purified lobster enolase and reference proteins on Sephadex G-100. The column was equilibrated and eluted with MI-buffer, at a flow rate of 27 ml/hr; 4-ml fractions were collected. Approximately 20 mg of each protein was applied to the column in a total volume of 5 ml. The proteins (MW given in parentheses) were: A, rabbit muscle aldolase (160,000); BSA, bovine serum albumin (69,000); O, ovalbumin (43.500); YE, yeast enolase (88,000); and LE, lobster enolase. The logarithm of the molecular weight is plotted as a function of the elution volume. Lobster enolase in two separate experiments eluted at 190 and 192 ml, corresponding to molecular weights of 84,000 and 90,000.

TABLE 1. Purification of lobster enolase.[a]

Fractionation step	Total activity (*units*)[b]	Total protein (*mg*)	Sp. Act. (*μ/mg*)	Recovery (%)
1. Extract	26600	4.15	6.5	100
2. 35–55% Acetone	31000	1.30	24	–
3. Heat supernatant	22800	0.605	38	86
4. 45–80% $(NH_4)_2 SO_4$ fraction	20100	0.54	37	77
5. First gel filtration eluate	17500	0.053	330	66
6. Second gel filtration eluate	11000	0.021	525	41
7. DEAE chromatography	5100	0.009	565	19

[a]Data are taken from a typical isolation procedure from 50 g of fresh muscle.
[b]The amount of enzyme that will give an absorbance change of 0.1 per min at 240 mμ.

Discussion

The purification procedure employed here is a combination of steps used in the past for the rabbit muscle enzyme (Holt and Wold 1961) and the trout enzyme (Cory and Wold 1966) incorporating the gel filtration and DEAE steps from the recent isolation of *Escherichia coli* enolase (T. G. Spring personal communication). The lobster enolase obtained by this procedure is 95–100% pure based on the acrylamide gel patterns.

The preliminary studies of the catalytic properties of lobster enolase gives further weight to the proposition that the enolase active site has remained remarkably constant through evolution. If one can interpret the data in Table 2 as indirect measures of the total makeup of the active site of enolase, there is no major difference between the enzymes from fish, from rabbit, and from lobster. Enolase from yeast shows a considerably elevated *p*H optimum compared with the other species. It may be significant that the *E. coli* enzyme also has a *p*H optimum at 7.8 (T. G. Spring personal communication). Thus, based on the *p*H optimum, yeast and *E. coli* enolases are in a class distinct from those of other species.

The physical and chemical properties of lobster enolase also concur quite well with those of the other enolases. There is only one apparently unique feature of the lobster enzyme, the cold sensitivity. Simply freezing and thawing once is enough to completely destroy the activity of this enzyme, whereas all the other enolases are frozen routinely and can be lyophilized without any significant loss in activity. It is also of interest that lobster enolase appears to exist in a single molecular form. This is in contrast to the salmonoid enolases that all have three enolase isozymes in characteristic proportions (Cory and Wold 1966; Ruth et al. 1970; Tsuyuki and Wold 1964).

TABLE 2. Activity characteristics of enolase from lobster and other sources.

Enzyme source	Km[a] (*moles/liter*)	Vmax[a] (*moles substr./min per mole of enzyme*)	*p*H optimum	Mg optimum
Lobster	1×10^{-4}	8700	6.95	10^{-3}
Rabbit[b]	0.6×10^{-4}	10000	6.90	10^{-3}
Trout[b]	0.4×10^{-4}	8000	6.90	10^{-3}
Yeast[b]	1×10^{-4}	11000	7.70	10^{-3}

[a]Determined at the optimal *p*H and Mg concentration.
[b]Data from Malmström (1961).

Acknowledgments

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Effects of Salinity, Temperature, and Dissolved Oxygen on Early Development of the Pacific Cod (*Gadus macrocephalus*)

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Effects of various combinations of levels of salinity, temperature, and dissolved oxygen on incubation of Pacific cod (*Gadus macrocephalus*) eggs were investigated in the laboratory. Quantitative relations were determined, by response surface analysis, between the environmental variables and four criteria used to estimate success of egg development. Larval size at mean hatching time, and percentages of total hatch, viable hatch, and postmature eggs that failed to hatch were optimized at intermediate salinities (about 15‰), low temperatures (3–5 C), and over a wide range of dissolved oxygen concentrations. Pacific cod eggs are judged to be euryhaline, euryoxic, and stenothermal. As long as temperatures are within the range of about 3–5 C, the developing egg is tolerant of a wide range of salinities, and of dissolved oxygen levels to a minimum of about 2 or 3 ppm. Below 3 C developmental success decreases precipitously; above 5 C it attenuates more slowly.

Distribution and abundance of Pacific cod are reviewed in light of the experimental results. Temperature is suggested to be of major importance to successful egg development in the natural environment; the temperature range associated with reasonable egg viability and incubation success is estimated to extend from about 2.5 to 8.5 C.

The distribution of cod in the spawning season appears associated with water temperatures between 2.5 and 8.5 C in Asian waters. Near the southern limit of cod distribution on the North American coast the following associations are suggested in relation to temperatures of the upper mixed layer of coastal waters in the spawning period: limit of commercial abundance, 9 C; extent of limited commercial landings, 10 C; limit of occurrence, 13.5 C.

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ANNUAL variations and geographic differences in oceanographic conditions prevailing during the early developmental stages of marine teleosts may contribute to changes in egg and larval survival, and consequently to the strength of year-classes produced.

The Pacific cod (*Gadus macrocephalus*)[1] occurs around the rim of the North Pacific Ocean and is subject to a considerable range of oceanographic conditions. Moiseev (1953) concluded that Pacific cod inhabited waters within a temperature range of −1.5–18 C, although they were better adapted to a more limited range between 0 and 10 C. Field evidence (Moiseev 1953; Ketchen 1961) indicated that distribution, abundance, and the short but intensive feeding migrations (Svetovidov 1948; Moiseev 1953) of Pacific cod are strongly related to cyclic changes in oceanographic conditions, particularly temperature. Pacific cod also exhibit seasonal bathymetric spawning and feeding migration cycles that are related to regional oceanographic characteristics and may occupy waters of considerable depth in the spawning period (Moiseev 1953; Ketchen 1961).

The Pacific cod egg is demersal (Thomson 1963). Estimates of the density of fertilized eggs yield a value of about 1.050 (1.052: Yamamoto and Nishioka 1952; 1.0494: Tamura 1952; 1.049: Forrester and Alderdice 1966), indicating that the eggs must incubate at the ocean floor. Thus, the development of Pacific cod eggs in deeper waters could be influenced by the availability of dissolved oxygen.

[1]In North American literature the Pacific cod is given specific rank (*Gadus macrocephalus*); others (Svetovidov 1948) recognize it as a subspecies of *Gadus morhua* (*G. m. macrocephalus*). In spite of the problem of nomenclature it is generally accepted that there is only one representative of the genus *Gadus* in the North Pacific Ocean (Ketchen 1961).

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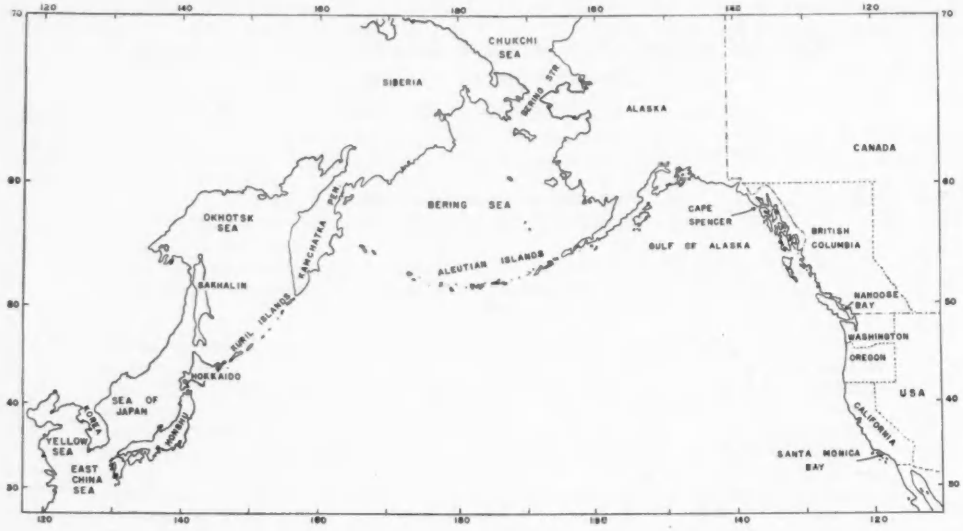

FIG. 1. The North Pacific Ocean, showing places referred to in the text.

The range of temperature tolerance of the poikilotherm egg is recognized as being narrower than that of the juvenile or adult stage. Furthermore, developmental response to temperature may be modified by salinity and dissolved oxygen levels. Forrester and Alderdice (1966) have concluded that cod eggs were stenothermal and euryhaline. The present experimental study was designed to investigate the effects of various combinations of salinity, temperature, and dissolved oxygen on egg development. Results are compared with field observations in an effort to indicate some of the environmental conditions that may govern egg survival under natural conditions.

DISTRIBUTION AND ECOLOGY

The geographic range of the Pacific cod extends from at least the northern part of the Yellow Sea (39°N, 123°E) (Svetovidov 1948) around the rim of the northern Pacific to Santa Monica Bay, California (34°N, 118°30′W) (Pinkas 1967). It inhabits the Bering Sea but does not extend into the Chukchi Sea (Alverson MS 1967; Pruter and Alverson 1962). The commercial range extends from the southern Sea of Japan region (Ministry of Agriculture and Forestry 1958, 1959, 1960, 1961, 1962) around the continental shelf to southern Washington State and, in some years, northern Oregon (Ketchen 1961; Pacific Marine Fisheries Commission 1968). In North American waters substantial stocks have been found in the southeastern Bering Sea and southward along the continental shelf to the United States–Canada border (Cobb 1927; International Pacific Halibut Commission 1964; Hitz and Rathjen 1965; Alverson MS 1967).

In Canadian coastal waters spawning concentrations are found from January to March at depths of about 100–135 m (Ketchen 1961) and this is followed by a movement to shallow water (about 50–75 m). Subsequently Pacific cod return to the deeper water during July–October (Alverson 1960; Ketchen 1961; Forrester and Prest MS 1968). The range in bathymetric distribution is thus from about 50 to 135 m. In Asian waters cyclic movements of cod between shallower and deeper regions are more pronounced (Ketchen 1961 fig. 8). Peak spawning in northern Asian waters (west Kamchatka etc.) occurs at depths of 80–290 m between February and April (Moiseev 1953 table 53). However, in the southern part of the Asian range the seasonal bathymetric movement is reversed. In the southern Kuril Islands, on the shores of Hokkaido and the eastern shores of Korea, Pacific cod move inshore to spawn in 15–50 m between December and February. After the spawning period these cod move offshore in March and April and remain at depths of 100 m or more during the summer period (Moiseev 1953). Thus, in Asian waters cyclic changes in bathymetric distribution (from about 15 to 290 m)

are of greater amplitude than in Canadian waters. Temperature changes in the upper mixed layer are much less pronounced in Canadian coastal waters than in Asian waters and are confined approximately to the upper 50 m (Dodimead 1961 and personal communication).

As a general rule, Pacific cod appear to spawn below the mixed layer at depths governed by its thickness. In most Asian waters, temperatures at these depths in the spawning period range between 0 and 5 C; the comparable range in Canadian waters is 6–9 C. Near the southern boundary of Asian distribution, where spawning occurs in shallow water, cod move into the shallows prior to spawning when waters have cooled to temperatures below 12–13 C. However, spawning does not occur until temperatures have dropped to 5–9 C or less (Moiseev 1953).

After spawning, the adult cod undertake feeding migrations to waters with temperatures from −1.3 to 10 C, although most adults are found at temperatures between 0.2–2.0 and 7–9 C. In some instances adult cod appear not to enter shallow water to feed until water temperatures have warmed above 0 C. After the intense postspawning feeding excursions that occur in Asian waters, cod move offshore when temperatures of the warming inshore region reach 10–12 C (Moiseev 1953).

From the foregoing we can assume that adult cod neither enter nor spawn in areas in which available temperatures remain above 12–13 C, and that spawning is probably inhibited at temperatures above 9 C or below 0 C. Therefore as the distances involved in the migration of Pacific cod tend to be short (Svetovidov 1948; Moiseev 1953), it is presumed that centers of abundance would be found where favorable spawning conditions, and in particular favorable incubation temperatures, exist.

Experimental Procedure

Live Pacific cod were obtained on two occasions for these studies (February 24, 1966; February 23, 1968), and were taken by trawl net from a spawning ground at Nanoose Bay, Strait of Georgia, B.C. (49°15′N, 124°W). Fish were taken at a depth of 77–88 m at a bottom temperature of 8.2 C (1966) and transported to the Biological Station where eggs were stripped and fertilized. Salinity of water used at fertilization was 26.44‰ (1966) and 27.91‰ (1968), at a temperature of 7.5 C. Egg diameters were as follows:

Year	Range (*mm*)	Mean (*mm*)	Hr after fertilization
1966	0.95–1.07	1.00	2¼
1968	0.93–1.07	0.98	2

Measured volumes of eggs were dispensed into incubation chambers within 1 hr after fertilization. Each incubator was held throughout the period of incubation at one of a total of 18 different sets of constant salinity, temperature, and dissolved oxygen conditions. Incubators were held in 35-liter insulated plastic tanks and were perfused by water from the tank in which each incubator was submerged (Alderdice and Forrester 1968; Alderdice and Velsen 1968). Constant pressure perfusion velocities of 235 cm/hr were obtained by using peristaltic action pumps, pulse columns, throttled delivery lines, and controlled-head overflows to each incubator.

The initial experimental design (1966) was a $2^k + 2k + 1$ orthogonal composite factorial array (Box 1956) with $k = 3$ environmental variables — salinity, temperature, and dissolved oxygen. Fifteen sets of incubation conditions were set up based on the initial design (Table 1). The design was extended (1968) by the addition of four further trials; one of these (trial 16) repeated a trial from the initial design (trial 9) (Table 1). Four subsamples of eggs were held under each incubation condition. The number of eggs in all subsamples average 272 (range 167–389) (Table 1).

Test waters employed (Table 2) were prepared by diluting 40‰ S salt water (with fresh water) to desired salinities; the concentrated salt water was prepared by mixing available sea water (27–29‰ S) and synthetic sea salt. Tank temperatures were controlled with regulated heaters and cooling coils. Dissolved oxygen concentrations were maintained by equilibrating the water in each tank with controlled mixtures of nitrogen gas and compressed air or oxygen-enriched compressed air. Test waters were renewed manually every 4 days. The four subsamples of eggs in each incubator were perfused in series (Alderdice and Velsen 1968); the inflow tube to each incubator was rotated through one subsample daily to average out possible bias resulting from subsample position.

Initially observations of eggs were made once daily in each incubator. Observations were increased to 6-hr intervals about 24 hr prior to the expected start of hatching, and maintained until hatching was complete. Hatched larvae were removed from the four cells of each incubator and counted every 6 hr. For each time interval the following data were obtained: (*a*) samples of total length measurements; abnormal larvae, usually with marked body curvature, were not measured; (*b*) total number of larvae hatched; (*c*) number of viable larvae, those deemed to be normal in appearance and behaviour. Distributions of hatching frequency were provided by the interval counts of hatched larvae. From these data the length of the incubation period (time from fertilization to mean of hatching frequency) was determined for each trial. In addition, a number of "postmature unhatched eggs" occurred in each trial. These eggs developed full-term embryos, but did not hatch. In most instances the embryos continued to develop within the capsule until the yolk was absorbed; their numbers appeared to be related to conditions imposed throughout incubation. Although they are treated

TABLE 1. Experimental conditions and numbers of eggs employed in the 15 trials of the initial design (1966) and for the four trials added later (1968).

Trial	Factors: Salinity (‰)	Temp (C)	Dissolved O_2 (ppm)	Eggs in each subsample: 1	2	3	4
			1966				
1	14	2.7	3.60	283	245	235	320
2	14	2.7	8.60	325	283	207	270
3	14	9.3	3.60	240	229	349	385
4	14	9.3	8.60	314	297	298	244
5	26	2.7	3.60	217	316	243	224
6	26	2.7	8.60	292	316	301	264
7	26	9.3	3.60	262	263	277	290
8	26	9.3	8.60	293	307	181	167
9	20	6.0	6.10	259	296	277	333
10	12.71	6.0	6.10	268	341	289	350
11	27.29	6.0	6.10	230	214	370	265
12	20	2.0	6.10	230	233	175	236
13	20	10.0	6.10	389	247	226	292
14	20	6.0	3.08	293	271	258	220
15	20	6.0	9.14	247	173	259	322
			1968				
16	20	6.0	6.10	475	518	493	426
17	19.28	5.43	12.40	353	420	312	400
18	16.77	5.75	9.46	472	845	784	824
19	14.25	6.05	6.53	375	425	406	473

TABLE 2. Test conditions achieved in the experimental trials.

Trial	Test conditions — (mean ± 1 SE): Salinity (‰)	Temp (C)	Dissolved O_2 (ppm)
1	14.03±0.01	2.74±0.02	3.59±0.07
2	14.01±0.01	2.73±0.03	8.55±0.04
3	14.03±0.02	9.32±0.02	3.46±0.04
4	14.01±0.02	9.34±0.03	8.53±0.04
5	26.03±0.01	2.72±0.04	3.54±0.03
6	26.02±0.01	2.73±0.03	8.39±0.06
7	26.00±0.02	9.31±0.01	3.47±0.05
8	26.01±0.01	9.33±0.02	8.50±0.04
9	20.02±0.01	6.02±0.04	6.04±0.11
10	12.70±0.01	6.03±0.02	6.11±0.03
11	27.28±0.02	6.00±0.03	5.97±0.03
12	20.03±0.01	2.03±0.02	6.03±0.03
13	20.03±0.02	10.04±0.02	6.08±0.04
14	20.05±0.01	6.05±0.02	3.05±0.03
15	20.04±0.01	6.01±0.01	9.06±0.03
16	19.99±0.04	6.00±0.01	6.08±0.01
17	19.29±0.04	5.46±0.00	12.45±0.02
18	16.76±0.03	5.76±0.01	9.42±0.01
19	14.26±0.03	6.05±0.01	6.54±0.03

separately, postmature unhatched eggs ultimately were classified as dead eggs.

For all of the trials the means of larval length and the percentages of total and viable larval hatches and postmature unhatched eggs were analyzed by response surface techniques (Box 1956). Regression surfaces were calculated showing the relation between each response variable and levels of the three environmental variables. The data were analyzed in two stages, employing as a linear model the polynomial

$$Y = b_0x_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$$

and as a nonlinear model

$$Y^\gamma = b_0x_0 + b_1x_1^{\alpha_1} + b_2x_2^{\alpha_2} + b_3x_3^{\alpha_3} + b_{11}x_1^{2\alpha_1} + b_{22}x_2^{2\alpha_2} + b_{33}x_3^{2\alpha_3} + b_{12}x_1^{\alpha_1}x_2^{\alpha_2} + b_{13}x_1^{\alpha_1}x_3^{\alpha_3} + b_{23}x_2^{\alpha_2}x_3^{\alpha_3}$$

where Y = measured response; x_1, x_2, x_3 = environmental variables salinity, temperature, and dissolved oxygen, respectively; γ, α_1, α_2, α_3 = power parameters, estimated by maximum likelihood procedure (Lindsey et al. 1970; Lindsey and Sandnes MS 1970). Canonical analysis has been utilized as a convenient method for

interpreting response domain configuration, and for calculating points for plotting two-dimensional surfaces on sections cut through the domain. Although the surfaces fitted are based on empirical models, they provide an appreciation of how environmental factors, acting in concert, determine or influence a biological response. In this respect the nonlinear model often provides a more adequate fit of a surface to the data, given the data and either the linear or the nonlinear model.

Results

Rate of Development

Length of the incubation period in the experimental trials varied between 10.0 days (trial 7) and 26.8 days (trial 12) (Table 3). Hatching frequency distributions were slightly skewed in most trials, there being a tendency for hatching frequency to rise abruptly, reach a maximum, and attenuate more slowly.[2] In general, 80–90% of the eggs in most trials hatched within a 45-hr period around the mean hatching time. At low salinities (at or below 14‰) a departure from this pattern involved a prolonging of the hatching interval.

[2]A square root transformation was applied to distributions of hatching frequency prior to the derivation of mean hatching times. The transformed time scale provided reasonably normal hatching frequency distributions.

Rate of development (reciprocal of incubation time × 100) of eggs in all trials is in substantial agreement with data from other sources (Fig. 2). The relation between rate of development and incubation temperature, irrespective of differences in salinity and dissolved oxygen levels, is approxi-

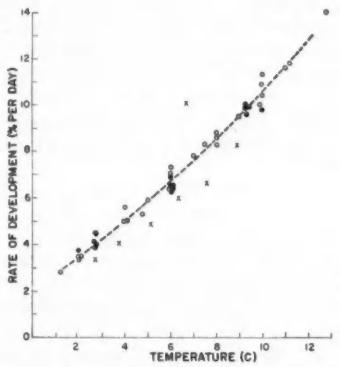

FIG. 2. Relation between temperature and rate of development of Pacific cod eggs. Solid circles — current study; open circles — data from other sources; ×'s — data from other sources considered here as outliers (other sources: Forrester and Alderdice 1966 appendix table I).

TABLE 3. Incubation time and the percentage of total hatch and viable hatch for subsamples of all of the trials.

Trial	Factors S (‰)	Temp (C)	O_2 (*ppm*)	Incubation time (*days*)	% total hatch, subsample 1	2	3	4	Mean	% viable hatch, subsample 1	2	3	4	Mean
1	14	2.7	3.60	24.19	78.80	73.06	67.66	56.56	68.51	65.72	60.41	45.96	42.19	53.28
2	14	2.7	8.60	25.74	70.77	83.39	82.13	65.56	74.93	62.46	70.67	64.73	47.41	61.29
3	14	9.3	3.60	10.41	65.83	70.74	66.76	76.36	70.41	57.50	61.14	62.18	64.16	61.68
4	14	9.3	8.60	10.14	58.92	71.72	32.21	29.92	49.18	54.46	65.99	24.50	27.05	43.89
5	26	2.7	3.60	22.22	1.84	1.27	0.41	0.89	1.10	0.00	0.00	0.41	0.00	0.10
6	26	2.7	8.60	24.89	48.63	58.54	52.49	51.89	53.03	44.86	55.06	46.18	46.21	48.25
7	26	9.3	3.60	10.00	6.87	6.46	12.64	14.83	10.35	5.34	6.08	11.19	14.48	9.43
8	26	9.3	8.60	10.09	24.91	49.19	37.02	26.35	35.34	22.53	44.30	35.36	25.75	32.59
9	20	6.0	6.10	15.88	84.94	75.34	85.56	84.08	82.40	83.40	75.00	84.12	83.18	81.37
10	12.71	6.0	6.10	14.37	82.46	81.52	67.82	83.71	79.17	74.63	75.37	59.86	75.14	71.55
11	27.29	6.0	6.10	15.29	19.57	28.50	65.41	51.70	44.95	19.13	27.10	64.32	51.32	44.11
12	20	2.0	6.10	26.76	8.26	3.86	5.71	2.54	5.03	1.74	2.15	2.86	1.27	1.95
13	20	10.0	6.10	10.18	33.16	39.27	52.21	41.44	40.29	25.96	27.13	49.12	37.67	33.71
14	20	6.0	3.08	15.31	63.48	78.60	64.73	80.91	71.40	60.41	76.01	60.08	77.73	68.04
15	20	6.0	9.14	15.47	88.66	91.33	91.12	85.09	88.61	86.23	87.86	91.12	83.85	87.01
16	20	6.0	6.10	15.39	56.00	79.92	54.77	87.79	69.25	48.63	72.20	49.70	63.03	62.29
17	19.28	5.43	12.40	17.36	55.52	81.43	83.65	70.25	72.73	43.63	74.76	69.87	64.00	63.43
18	16.77	5.75	9.46	15.09	91.10	86.04	81.25	88.96	86.39	86.02	80.36	71.94	79.49	78.77
19	14.25	6.05	6.53	15.32	85.07	83.53	73.89	82.24	81.18	76.00	76.71	65.27	72.30	72.54

mated by the trend line in Fig. 2 (omitting the indicated outliers); hence

$$Y = 2.2510 + 0.5899x + 0.0243x^2$$

where Y = rate of development, percent/day; and x = incubation temperature, C. The effect of all three environmental variables on rate of development is estimated by fitting a polynomial to the estimates of incubation time for all trials (Table 3). Hence

$$Y = 32.479 + 0.145x_1 - 4.404x_2 + 0.305x_3 - 0.009x_1^2 + 0.200x_2^2 - 0.011x_3^2 + 0.015x_1x_2 + 0.018x_1x_3 - 0.066x_2x_3$$

where Y = incubation time, days; and x_1, x_2, x_3 = salinity (‰), temperature (C), and dissolved oxygen (ppm) respectively. Corresponding rates of development predicted for various salinity, temperature, and dissolved oxygen combinations are compared in Fig. 3; rate of development shows only a limited dependence on salinity and dissolved oxygen levels within the ranges employed.

Size of Larvae

The average length of newly hatched larvae tended to increase in successive samples taken through the initial part of the hatching period. At higher incubation temperatures, mean larval length reached a maximum at or shortly after the time of maximum hatching frequency and declined in subsequent samples (Fig. 4, trial 4). In the low temperature trials (2.0, 2.7 C) average larval size tended to increase throughout the hatching period (Fig. 4, trial 6). In these latter instances the fewer but larger larvae produced tended to be larger than those produced at mean hatching time under higher temperatures. Larval size relations suggested by the data from all of the trials are indicated by the model shown in Fig. 5.

The relation between mean hatching time and larval size was considered further in attempting to relate larval size to the conditions under which the eggs were incubated. Three ways of expressing larval size were considered, namely (*a*) average size of all larvae sampled, (*b*) maximum size at-

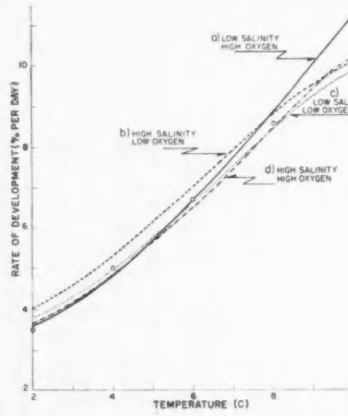
a) LOW SALINITY HIGH OXYGEN
b) HIGH SALINITY LOW OXYGEN
c) LOW SALINITY LOW OXYGEN
d) HIGH SALINITY HIGH OXYGEN
RATE OF DEVELOPMENT (% PER DAY)
TEMPERATURE (C)

FIG. 3. Predicted effects of several salinity–dissolved oxygen combinations ((*a*) 14‰, 8.60 ppm; (*b*) 26‰, 3.60 ppm; (*c*) 14‰, 3.60 ppm; (*d*) 26‰, 8.60 ppm) on rate of development of Pacific cod eggs over the temperature range investigated (2–10 C). Open circles indicate the trend for the data marshalled in Fig. 1.

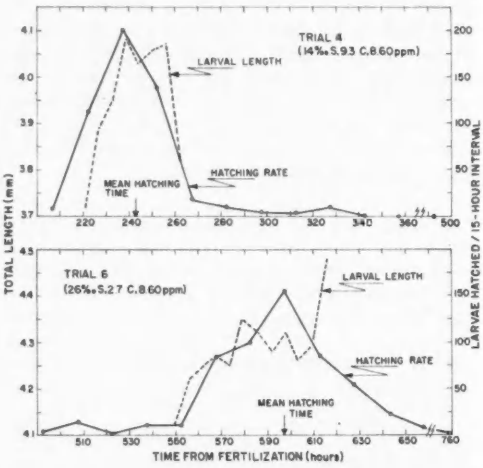

FIG. 4. Relation between hatching rate and larval size for two selected high (9.3 C) and low (2.7 C) incubation temperatures.

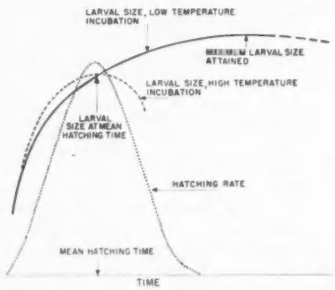

FIG. 5. Model employed to suggest the relation between hatching rate and larval size for all experimental trials.

tained in successive samples, and (*c*) larval size at the mean hatching time. The latter was chosen as the most meaningful, as it reflects the size class attained at hatching by the majority of larvae incubated under the conditions imposed. Estimates of larval size at mean hatching time (Table 4) were calculated by regression, fitting a polynomial to means of larval length obtained from successive samples for each trial under the assumptions illustrated in Fig. 5.

Using the estimates of larval size at mean hatching time, those levels of salinity, temperature, and dissolved oxygen may be calculated that maximize this measure of size. Analysis was conducted using response surface techniques. The adequacy of both the linear and nonlinear models was tested (Lindsey and Sandnes MS 1970) the better fit being given by the former. Hence

$$Y = 3.692 + 0.0324x_1 + 0.1194x_2 + 0.0052x_3 - 0.0008x_1^2 - 0.0094x_2^2 - 0.0016x_3^2 - 0.0013x_1x_2 + 0.0014x_1x_3 - 0.0015x_2x_3$$

where Y = larval length, mm; and x_1, x_2, x_3 = salinity (‰), temperature (C), and dissolved oxygen (ppm) respectively. Analysis of variance of these data, and partitioning into components associated with regression on x_1, x_2, x_3, x_1^2, x_2^2, x_3^2, x_1x_2, x_1x_3, and x_2x_3, indicates that all but the x_2 and x_2^2 terms associated with temperature may be eliminated. Hence, the effect of temperature on larval size may be approximated by

$$Y = 3.692 + 0.1194x_2 - 0.0094x_2^2$$

where Y = larval size, mm; and x_2 = temperature, C.

Retaining all terms of the fitted polynomial as the best available estimates, regression surfaces may be calculated to describe the relation between larval size and combinations of levels of salinity, temperature, and dissolved oxygen. Reducing the polynomial to canonical form (Box 1956),

$$Y - 4.36 = -0.000318Z_1^2 - 0.009471Z_2^2 - 0.001973Z_3^2$$

where Y = larval size, mm; Y_s = 4.36 mm, the value of Y at *s*, the centre of the surface; and Z_1, Z_2, Z_3 = canonical variables (linear combinations of the environmental variables). The signs of the terms on the right-hand side of the equation are all negative, indicating that the response domain is closed (ellipsoidal) with larval size a maximum at its centre *s*. The centre is calculated to occur at or near

s = (26.44‰ S, 3.61 C, 11.2 ppm dissolved oxygen).

Surfaces sliced through *s* in the three environmental factor planes indicate the relations between salinity, temperature, and dissolved oxygen levels throughout incubation and their influence on larval size at mean hatching time (Fig. 6). At the centre of the three surfaces maximum larval size is associated with moderately high salinity, low temperature, and high dissolved oxygen levels (at 1 atmosphere pressure, 26.4‰ S, and 3.6 C, water is saturated at about 10.8 ppm O_2). Temperature has a dominant influence on larval size. Larval size decreases rapidly at temperatures above 3.61 C (at *s*), although the range of near-maximum sizes occurs over a broad range of salinity (Fig. 6*a*) and dissolved oxygen (Fig. 6*c*) levels. The extent of these ranges is shown in the salinity–dissolved oxygen surface (Fig. 6*b*).

TABLE 4. Calculated total length of larvae (mm) at mean hatching time based on samples of larvae taken throughout the hatching period.

Trial	Total length (*mm*)
1	4.23
2	4.29
3	4.12
4	4.09
5	..
6	4.33
7	4.00
8	4.07
9	4.42
10	4.28
11	4.32
12	4.27
13	4.10
14	4.31
15	4.28
16	4.26
17	4.33
18	4.21
19	4.29

SURVIVAL TO HATCHING

Table 3 lists the percentages of eggs that hatched (total hatch), and the percentages of eggs that produced normal larvae (viable hatch). Preliminary analysis of the first set of trials (1966) indicated that further tests would be required to better define optimum conditions for development. Accordingly four further trials (1968) were completed on a

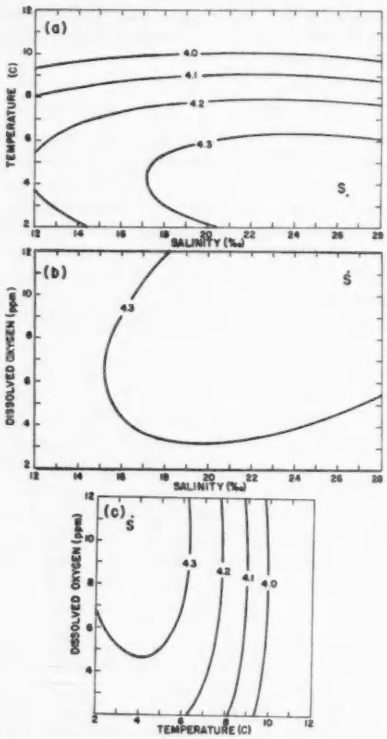

FIG. 6. Regression surfaces for larval size as a function of levels of salinity, temperature, and dissolved oxygen. Three planes are shown in the test space: (*a*) salinity–temperature, (*b*) salinity–dissolved oxygen, and (*c*) temperature–dissolved oxygen. Maximum larval size is calculated to occur at or near *s*, the common centre of the surfaces. Isopleths of larval size, mm, are shown around the center *s*.

linear path through the salinity–temperature–dissolved oxygen test space (Box 1956 p. 502) in the estimated direction of maximum total hatch. Analysis was completed on the combined results.

Total hatch

Over the ranges of the environmental variables examined, total hatch was depressed markedly by low temperatures (trial 12; 20‰ S, 2.0 C, 6.10 ppm O_2) and by combined low temperature and low dissolved oxygen levels (trial 5; 26‰ S, 2.7 C, 3.60 ppm O_2). In the latter instance, however, a reduction in salinity substantially increased survival to hatching (trial 1; 14‰ S, 2.7 C, 3.60 ppm O_2). Highest total hatches were associated with moderate salinities (12.71–20‰), medium temperatures (6.0 C), and low to high dissolved oxygen levels (3.08–9.14 ppm).

Further analysis was undertaken to predict the effects in the test space of the three environmental factors acting in concert. Initially the linear model was employed. This model, the basis for earlier interpretations (Forrester and Alderdice 1966; Alderdice and Forrester 1968), was compared with and rejected in favour of the nonlinear model. In the latter instance the power parameters associated with the dependent (response) and independent (enrivonmental) variables are free (from the constraint that the power parameters are unity as in the linear case) to assume their maximum likelihood values (Box and Tidwell 1962; Lindsey et al. 1970). Hence for total hatch

$$Y^{0.54} = 46.79 + 1.85 \times 10^{-5}x_1^{3.72} + 95.35x_2^{-0.95} + 85.31x_3^{-2.16} - 6.89 \times 10^{-11}x_1^{7.44} - 160.6x_2^{-1.90} - 3.08\,x_3^{-4.32} - 3.04 \times 10^{-5}x_1^{3.72}x_3^{-0.95} - 6.93 \times 10^{-4}\,x_1^{3.72}x_3^{-2.16} - 230.0x_2^{-0.95}x_3^{-2.16}$$

where Y = percent total hatch; x_1, x_2, x_3 = salinity (‰), temperature (C), and dissolved oxygen (ppm), respectively; and α_1, α_2, α_3, γ = 3.7197,– 0.9455, –2.1640, and 0.5443, respectively. The relative likelihood (R) of the linear model adequately representing the data, as opposed to the nonlinear model, is 5.65×10^{-13}, or nearly zero.

Analysis of variance associated with total hatch is given on the basis of the nonlinear model (Table 5). Variance is partitioned into components associated with treatment effects (19 trials) and an error term. The treatment component was further partitioned into components associated with regression on $x_1^{\alpha_1}$, ..., $x_{11}^{\alpha_{11}}$, ..., $x_2^{\alpha_2}x_3^{\alpha_3}$ and a component not explained by regression on these terms (lack of fit).

In the analysis of the nonlinear case, standard F tests are not valid as the probabilities for the ratios of sums of squares cannot be read from existing tables. Hence the fifth column in Table 5 gives an approximation of the variance ratio, F. Exact inferences regarding the effects of the listed components may be found in the sixth column (MLR), which provides the relative likelihoods of the various α coefficients of the regression equation being zero, and of the power parameters (transform) being unity. A fuller explanation of the fitting of response surfaces using nonlinear models may be found in Lindsey et al. (1970) where the above data are used as an example. Examination of the MLR column indicates that treatment effects explain a large portion of the variance, that the power transformation markedly improves the

TABLE 5. Analysis of variance of the data for percent total hatch of Pacific cod eggs according to the nonlinear model.

Source of variance	SS	df	MS	Approx F	MLR
Treatments	68682.91	18	3815.71	43.86	0.00
Regression	67556.24	9	7506.24	76.47	0.00
Linear	30038.96	3	10012.98	102.01	4.04×10^{-20}
x_1	18525.03	1	18525.03	188.74	8.71×10^{-24}
x_2	6278.16	1	6278.16	63.96	2.00×10^{-12}
x_3	2846.79	1	2846.79	29.00	4.64×10^{-7}
Quadratic	22455.93	3	7485.31	76.26	3.12×10^{-20}
x_1^2	300.19	1	300.19	3.05	0.160
x_2^2	18725.30	1	18725.30	190.78	6.40×10^{-24}
x_3^2	0.01	1	0.01	0.00	0.999
Interaction	11992.50	3	3997.49	40.72	1.07×10^{-13}
$x_1 * x_2$	399.03	1	399.03	4.06	8.95×10^{-2}
$x_1 * x_3$	8547.99	1	8547.99	87.09	3.31×10^{-15}
$x_2 * x_3$	2323.15	1	2323.15	23.66	4.61×10^{-6}
Transform	5835.50	4	1458.87	14.86	5.65×10^{-13}
Residual	6085.30	62	98.14		
Lack of fit	1126.68	5	225.33	2.59	4.18×10^{-4}
Pure error	4958.62	57	86.99		
Total	73641.53	75			

fitted relation, and that the quadratic terms for salinity and dissolved oxygen and the salinity–temperature interaction term could be eliminated without greatly affecting the adequacy of the model.[3] The likelihood may also be examined of the parameters taking other values than their maximum likelihood estimates. Hence the maximized relative likelihood (R) of the power parameters taking the following discrete values is $R(\gamma = 0.5) = 1.0$, $R(\alpha_1 = 4.0) = 0.99$, $R(\alpha_2 = -1.0) = 0.89$, and $R(\alpha_3 = -2.0) = 0.99$. A simpler approximation of the effects of the three environmental variables on total hatch then would be (Lindsey et al. 1970)

$$Y^{0.5} = \beta_0 x_0 + \beta_1 x_1^4 + \beta_2 x_2^{-1} + \beta_3 x_3^{-2} + \beta_{22} x_2^{-2} + \beta_{13} x_1^4 x_3^{-2} + \beta_{23} x_2^{-1} x_3^{-2}.$$

For response surface analysis of these data the fitted nonlinear model is retained using the computed values of the parameters as the best available estimates. The canonical equation is

$$Y - 12.423 = -1.487 \times 10^{-9} Z_1^2 - 221.174 Z_2^2 + 57.562 Z_3^2.$$

The response at the centre of the domain is calculated as 102.4%[4] total hatch at

$$s = (15.59‰ \text{ S}, 3.73 \text{ C}, 9.03 \text{ ppm dissolved oxygen}).$$

The positive sign of the Z_3 term in the canonical equation indicates that higher response values may be found in the direction of the Z_3 axis. The Z_3 axis is associated primarily with changes in dissolved oxygen concentration. It is assumed that a better estimate of s would be obtained if further experimental trials were conducted at higher dissolved oxygen levels. However, the calculated center is considered a reasonable estimate of the true center, as the dissolved oxygen concentration

[3]Maximum likelihood ratios of 0.1 for the various parameters are assumed to border the likelihood that such parameters could be replaced by zero. For example, the likelihood that the x_1 term (Table 5) could be eliminated from the model is very small (8.71×10^{-24}). However, the x_1 quadratic term likelihood ratio is greater than 0.1 (0.160), suggesting that it could be eliminated. On the other hand, with reference to the term for lack of fit, if the fitted polynomial is compared with a higher degree polynomial using all of the available degrees of freedom, the better the fit the closer will the ratio of their likelihoods approach 1.0. For example the MLR value in the table (4.18×10^{-4}) suggests that a better fit could be obtained, even though the nonlinear model provides a superior fit to that obtainable using the linear model.

[4]If the response at the surface centre were predicted without error, its value presumably would be near but not greater than 100%.

at s is near saturation (about 11.65 ppm at 15.6‰ S and 3.7 C).

Contour plots for percent total hatch (Fig. 7) for the salinity–temperature, salinity–dissolved oxygen, and temperature–dissolved oxygen planes through the centre, s, indicate near-maximum percent total hatches occur over a wide range of salinity and dissolved oxygen levels, but are restricted to a narrow range of temperatures. For example, the 90% total hatch contour is bounded by salinities from 12.71‰ or less to about 24‰, at temperatures between 3 and 5 C (Fig. 7*a*). Total hatch declines rapidly at temperatures below 3 C, but more slowly above 5 C. Figure 7*b* indicates that near-maximum total hatch (for example 90% or greater) occurs at salinities below about 24‰ and dissolved oxygen levels above 3 ppm; at 3 ppm dissolved oxygen, salinities associated with near-maximum total hatches are below about 17‰. Total hatch declines rapidly at salinities below 17‰ when dissolved oxygen levels are below 3 ppm.

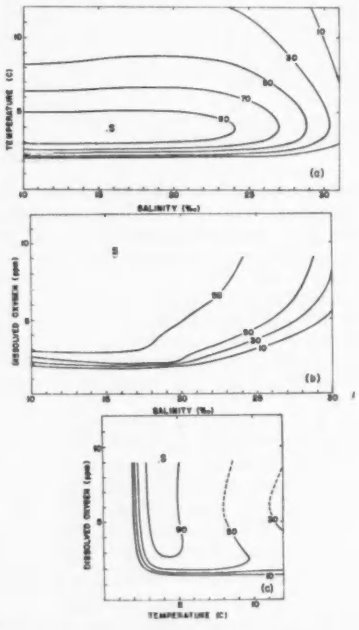

FIG. 7. Nonlinear regression surfaces for percent total hatch as a function of levels of salinity, temperature, and dissolved oxygen. Three planes are shown in the test space: (*a*) salinity–temperature, (*b*) salinity–dissolved oxygen, and (*c*) temperature–dissolved oxygen. Maximum total hatch is calculated to occur at or near s, the common center of the three environmental variable planes. Isopleths of percent total hatch are shown around s.

Figure 7*c* indicates that near-maximum total hatches are attained at dissolved oxygen levels of 3 ppm or more as long as temperatures are maintained between 3 and 5 C. The relation of the three surfaces to each other is shown in Fig. 8. In summary the data suggest that the Pacific cod egg is stenothermal, euryhaline, and euryoxic[5] in terms of total hatch, and that maximum total hatches are associated with salinities around 16‰, and dissolved oxygen concentrations near saturation at temperatures near 3 C.

Viable hatch

Effects of salinity, temperature, and dissolved oxygen on percent viable hatch correspond with the results obtained for total hatch (Table 3). Viable hatch, as in the previous instance, was considered as a function of the three environmental variables in terms of both linear and nonlinear response surface models. The relative likelihood of the linear model adequately representing the data, as opposed to the nonlinear model, is 1.11×10^{-12}. Hence for percent viable hatch

$$Y^{0.33} = 47.33 + 3.58 \times 10^{-7}x_1^{4.64} + 38.85x_2^{-0.98} + 39.49x_3^{-2.28} - 4.39 \times 10^{-14}x_1^{9.29} - 68.94x_2^{-1.95} + 5.74x_3^{-4.56} - 7.21 \times 10^{-7}x_1^{4.64}x_2^{-0.98} - 1.69 \times 10^{-6}x_1^{4.64}x_3^{-2.28} - 127.6x_2^{-0.98}x_3^{-2.28}$$

where Y = percent viable hatch; x_1, x_2, x_3 = salinity (‰), temperature (C), and dissolved oxygen (ppm), respectively; and α_1, α_2, α_3, γ = 4.6431, −0.9752, −2.2794, and 0.3827, respectively.

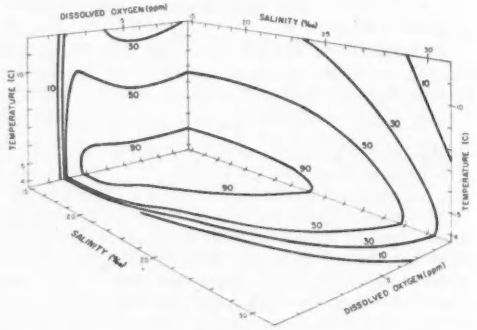

FIG. 8. The three planes of Fig. 7 are shown in relation to one another. The origin is at s = 15.59‰ S, 3.73 C, 9.03 ppm O_2.

[5]The term *euryoxic* is employed here to signify tolerance to a wide range of dissolved oxygen concentrations.

Analysis of variance of the data is given for the nonlinear model (Table 6) in the same manner as for total hatch. Examination of the MLR column of the table indicates that treatment effects explain a large portion of the variance, that the power transformation improves the fitted relation, and that the quadratic terms associated with salinity and dissolved oxygen could be eliminated without greatly affecting the adequacy of the model. However, as in the previous instance for total hatch, a substantial portion of the treatment variance is not explained by regression.

The relative likelihood (R) of the powers taking other values than their maximum likelihood estimates again may be examined. Thus, $R(\alpha_1 = \alpha_2 = \alpha_3 = \gamma = 1.0) = (0.05, 0.00, 0.00, 0.00)$. In addition, $R(\alpha_1 = 4.0) = 0.94$, $R(\alpha_2 = -1.0) = 0.98$, $R(\alpha_3 = -2.0) = 0.97$, and $R(\gamma = 0.50) = 0.37$. Therefore a simplified model for viable hatch might be considered as

$$Y^{0.5} = b_0x_0 + b_1x_1^4 + b_2x_2^{-1} + b_3x_3^{-2} + b_{22}x_2^{-2} + b_{12}x_1^4x_2^{-1} + b_{13}x_1^4x_3^{-2} + b_{23}x_2^{-1}x_3^{-2}.$$

The canonical equation

$$Y - 5.75 = -1.0607 \times 10^{-12}Z_1^2 - 105.549Z_2^2 + 42.337Z_3^2$$

indicates that higher percent viable hatches should be found in the direction of the Z_3 axis, the axis primarily associated with changes in dissolved oxygen concentration.

Response at the provisional center of the surface, calculated as 96.60% viable hatch, is estimated to occur at or near

s = (14.91‰ S, 3.79 C, 8.28 ppm dissolved oxygen).

Higher dissolved oxygen concentrations on the Z_3 axis would be coupled with small salinity and temperature changes away from those at the provisional centre, s. At 14.9‰ S and 3.8 C, water would be saturated at about 11.7 ppm dissolved oxygen. On the Z_3 axis 11.7 ppm dissolved oxygen would be coupled with salinity and temperature levels of 14.91‰ and 3.75 C (calculated from the canonical equation). Therefore, it is assumed that the provisional maximum found is very near to the true center of the surface.

Contour plots for planes through the provisional center of the surface suggest that maximum and near-maximum viable hatches (90% or greater) would be found between salinities of 12.71‰ or less and about 23‰, when associated temperatures lie between 3 and 4.5 C (Fig. 9*a*). Near-maximum viable hatches would be expected within the stated salinity range at dissolved oxygen concentrations between about 2 ppm and saturation levels or greater (Fig. 9*b*). The latter figure indicates that viable hatch is dependent on an association between salinity and dissolved oxygen at salinities above

TABLE 6. Analysis of variance of the data for percent viable hatch of Pacific cod eggs according to the nonlinear model.

Source of variance	SS	df	MS	Approx F	MLR
Treatments	67918.26	18	3773.23	51.61	0.00
Regression	66117.07	9	7346.34	76.31	0.00
Linear	29324.18	3	9774.72	101.54	4.68×10^{-20}
x_1	13308.62	1	13308.62	138.25	4.48×10^{-20}
x_2	10198.08	1	10198.08	105.93	3.59×10^{-17}
x_3	3518.76	1	3518.76	36.55	2.25×10^{-8}
Quadratic	20531.80	3	6843.93	71.09	2.51×10^{-25}
x_1^2	112.91	1	112.91	1.17	0.491
x_2^2	16746.40	1	16746.40	173.96	8.77×10^{-23}
x_3^2	0.11	1	0.11	0.00	0.999
Interaction	13391.74	3	4463.91	46.37	3.80×10^{-20}
$x_1 * x_2$	516.51	1	516.51	5.36	4.27×10^{-2}
$x_1 * x_3$	9324.31	1	9324.31	96.86	2.97×10^{-16}
$x_2 * x_3$	2794.81	1	2794.81	29.03	4.59×10^{-7}
Transform	4810.70	4	1202.67	12.49	1.11×10^{-12}
Residual	5968.39	62	96.26		
Lack of fit	1801.19	5	360.23	4.92	1.18×10^{-6}
Pure error	4167.19	57	73.10		
Total	72085.46	75			

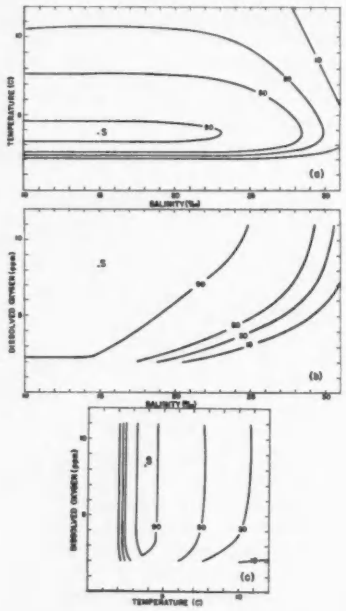

FIG. 9. Nonlinear regression surfaces for percent viable hatch. Three planes are shown in the test space: (*a*) salinity–temperature, (*b*) salinity–dissolved oxygen, and (*c*) temperature–dissolved oxygen. Other features are as indicated for Fig. 7.

approximately 15‰. At lower salinities viable hatch is near-maximal over the greatest range of dissolved oxygen concentrations; at higher salinities near-maximum response is associated with a more limited range of higher dissolved oxygen levels. Conversely at high dissolved oxygen levels, near-maximum viable hatches occur over the greatest range of salinities. Figure 9*c* shows that near-maximum viable hatches are found at temperatures between about 3 and 4.5 C where dissolved oxygen levels range from 2 to 3 ppm to saturated levels or greater. In other words, as long as temperatures are within the range of 3–4.5 C, near-maximum viable hatches are relatively independent of dissolved oxygen levels above 2–3 ppm. Figure 10 is a composite of the three surfaces described, assembled to show how the surfaces relate to one another in the viable hatch domain. The domain is very similar to that for percent total hatch (Fig. 8). It appears to be ellipsoidal, but in fact is "bowl-shaped," with its open top in the salinity–temperature plane on the oxygen axis at higher concentrations behind the plane of the paper.

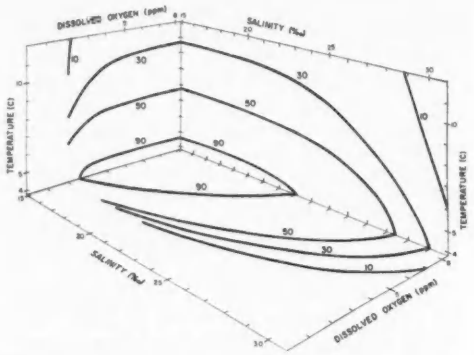

FIG. 10. The three planes of Fig. 9 are shown in relation to one another. The origin is at $s = 14.91‰$ S, 3.79 C, 8.29 ppm O_2.

In summary, in terms of viable hatch the Pacific cod egg appears to be euryhaline, euryoxic, and stenothermal. Maximum and near-maximum viable hatches are associated with temperatures between 3 and 4.5 C over a range of salinities from at least 12.71 to 23‰. At low salinities in this range near-maximum viable hatches occur over a range of dissolved oxygen levels from 2 or 3 ppm to saturation or greater; as salinity increases the range of dissolved oxygen levels associated with near-maximum viable hatch decreases, approaching saturation levels at the upper end of the salinity range.

Postmature unhatched eggs

The relation of percent postmature unhatched eggs to levels of salinity, temperature, and dissolved oxygen (Table 7) was examined in terms of both the linear and nonlinear response surface models. Difficulty was experienced in finding a unique set of maximum likelihood estimates for the power parameters in the nonlinear instance. One of the powers, α_3, was poorly defined leading to solutions involving more than one set of power parameters of nearly equal plausibility. These alternate sets generated quite comparable response surface configurations. One of these solutions is presented; the relative likelihood of the linear model adequately representing the data, as opposed to the nonlinear model, is 2.65×10^{-18}.

Hence, for postmature unhatched eggs

$$Y^{0.25} = 18.24 - 8.25 \times 10^{-2}x_1^{1.0} - 5.92x_2^{-1.0} - 6.07 \times 10^{-2}x_3^{1.5} + 4.89 \times 10^{-2}x_1^{2.0} + 13.19x_2^{-2.0} + 5.09 \times 10^{-4}x_3^{3.0} - 0.24x_1^{1.0}x_2^{-1.0} + 4.20 \times 10^{-4}x_1^{1.0}x_3^{1.5} + 0.14x_2^{-1.0}x_3^{1.5}$$

where Y = percent postmature unhatched eggs; x_1, x_2, x_3 = salinity (‰), temperature (C), and dissolved oxygen (ppm), respectively; and α_1, α_2, α_3, γ = 1.0, −1.0, 1.5, and 0.25, respectively.

Analysis of variance of the data (Table 8) indicates that treatment effects explain a large portion of the variance, that the power transformation provides a more plausible fit than the linear model, and that the x_2 and x_1x_3 terms could be eliminated without greatly affecting the adequacy of the model. Nevertheless deviations from regression indicate that although the fitted equation provides

TABLE 7. Percentage of eggs reaching full-term development, which subsequently did not hatch (postmature unhatched).

	Factors			% postmature unhatched, subsample				
Trial	S (‰)	Temp (*C*)	O_2 (*ppm*)	1	2	3	4	Mean
1	14	2.7	3.60	3.89	4.08	5.53	5.00	4.62
2	14	2.7	8.60	8.31	3.18	3.86	5.56	5.44
3	14	9.3	3.60	7.92	12.23	4.87	5.97	7.23
4	14	9.3	8.60	15.29	3.03	1.68	33.20	12.40
5	26	2.7	3.60	6.91	2.85	3.29	6.25	4.60
6	26	2.7	8.60	29.79	20.57	26.91	17.05	23.70
7	26	9.3	3.60	82.44	81.37	64.98	80.34	77.20
8	26	9.3	8.60	52.90	28.66	43.09	42.51	41.35
9	20	6.0	6.10	8.49	5.07	7.22	4.80	6.27
10	12.71	6.0	6.10	5.60	2.35	0.35	2.00	3.47
11	27.29	6.0	6.10	63.48	61.68	24.32	40.00	43.93
12	20	2.0	6.10	7.39	16.31	10.29	5.08	9.73
13	20	10.0	6.10	22.88	18.22	13.72	24.66	20.54
14	20	6.0	3.08	24.91	14.76	22.88	12.27	19.10
15	20	6.0	9.14	4.45	2.89	5.02	2.80	3.80
16	20	6.0	6.10	11.16	10.81	17.04	6.57	11.56
17	19.28	5.43	12.40	11.90	6.43	6.41	9.25	8.48
18	16.77	5.75	9.46	1.48	3.31	7.02	2.31	3.73
19	14.25	6.05.	6.53	3.20	3.53	5.67	4.65	4.29

TABLE 8. Analysis of variance of the data for postmature unhatched Pacific cod eggs according to the nonlinear model.

Source of variance	SS	df	MS	Approx F	MLR
Treatments	7794.97	18	433.05	20.78	4.06×10^{-24}
Regression	7074.06	9	786.00	25.94	2.74×10^{-26}
Linear	4024.47	3	1341.48	44.28	1.91×10^{-19}
x_1	3849.96	1	3849.96	127.08	5.95×10^{-19}
x_2	258.57	1	258.57	8.53	8.00×10^{-3}
x_3	0.00	1	0.00	0.00	0.999
Quadratic	880.59	3	293.53	9.68	5.48×10^{-7}
x_1^2	296.63	1	296.63	9.79	4.13×10^{-3}
x_2^2	725.35	1	725.35	23.94	4.83×10^{-6}
x_3^2	134.17	1	134.17	4.42	7.56×10^{-2}
Interaction	1182.18	3	394.05	13.00	1.11×10^{-6}
$x_1 * x_2$	632.41	1	632.41	20.87	1.89×10^{-5}
$x_1 * x_3$	8.98	1	8.98	0.29	0.837
$x_2 * x_3$	524.77	1	524.77	17.32	9.80×10^{-5}
Transform	2078.49	3	692.83	22.87	2.65×10^{-10}
Residual	1908.51	63	30.29		
Lack of fit	720.91	6	120.15	5.76	1.48×10^{-3}
Pure error	1187.60	57	20.83		
Total	8982.57	75			

a highly plausible model, other models exist that would provide a better fit. The linear term for salinity (x_1) accounts for a large portion of the treatment variance, and that all terms involving dissolved oxygen except the x_2x_3 interaction either satisfy only a small portion of the treatment variance or could be eliminated.

The canonical equation for the nonlinear model is

$$Y - 1.28 = 0.0040Z_1^2 + 13.2004Z_2^2 - 0.000076Z_3^2.$$

The equation indicates that a provisional centre of *minimum* response has been found. Lower percentages of postmature unhatched eggs would be found in the direction of the Z_3 axis, presumably at higher concentrations of dissolved oxygen. A calculated value of 2.70% postmature unhatched eggs is predicted at the centre of minimum response, at

s = (14.06‰ S, 3.87 C, 6.72 ppm dissolved oxygen).

Contour plots for postmature unhatched eggs are given in Fig. 11. At low temperatures (3–5 C) the eggs tolerate the widest range of salinities (Fig. 11*a*) and dissolved oxygen levels (Fig. 11*c*). At higher temperatures (above 5 C) the eggs require higher minimum dissolved oxygen levels (Fig. 11*c*) and lower salinities (Fig. 11*b*) to minimize the proportion of postmature unhatched eggs.

In summary, in terms of postmature unhatched eggs the Pacific cod egg appears to be euryhaline, euryoxic, and stenothermal. Minimum and near-minimum proportions of postmature unhatched eggs occur over a broad range of salinity and dissolved oxygen levels as long as temperatures are in the range of 3–5 C.

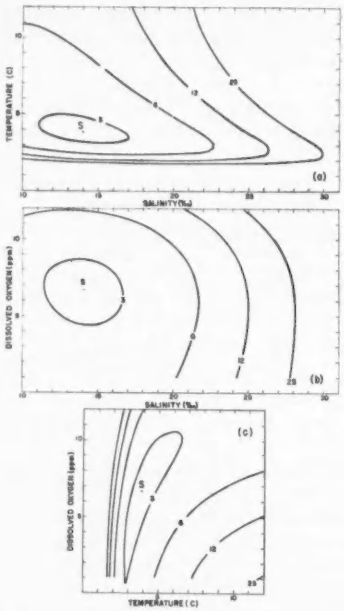

FIG. 11. Nonlinear regression surfaces for percent postmature unhatched eggs. Three planes are shown in the test space: (*a*) salinity–temperature, (*b*) salinity–dissolved oxygen, and (*c*) temperature–dissolved oxygen. A minimum occurs at the centre, *s*. Other features are as indicated in Fig. 7.

Discussion

The significance of the current laboratory findings may be reviewed with reference to conditions associated with the natural distribution of the Pacific cod. The experimental data are suggested as being true measures of developmental response of Pacific cod eggs to the environmental variables investigated. However, the natural environment is of far greater complexity than the test space within which the experimental results were obtained. Therefore, ecological inferences drawn from the experimental results should be considered as first approximations to the manner in which the environmental variables investigated act on Pacific cod through their effects on incubation and egg development in the natural environment.

Spawning and Early Egg Development

From the experimental evidence, optimum temperatures for the developmental criteria considered are consistently within the narrow range of 3.5–4 C (Fig. 12). These were computed as follows: maximum (*a*) larval size, 3.61 C; (*b*) total hatch, 3.73 C; (*c*) viable hatch, 3.99 C; and minimum (*d*) postmature unhatched eggs, 3.87 C. In addition, optimum responses associated with these temperatures occur over a broad range of salinities. The cod egg also appears to tolerate a wide range of dissolved oxygen levels from saturation down to 2–3 ppm, as long as temperatures are within the optimum range. The form of the response surface (Fig. 7*a*) indicates that egg survival at temperatures above 3.5–4 C decreases less precipitously than at temperatures below the surface center for total hatch. From the figure, total hatches of 50% or greater would be expected at temperatures between about 2.5 and 8.5 C.

It is surprising that three of the four response centers (Fig. 12) occur at lower salinities (between 14 and 16‰) and that the isopleths of near-maxi-

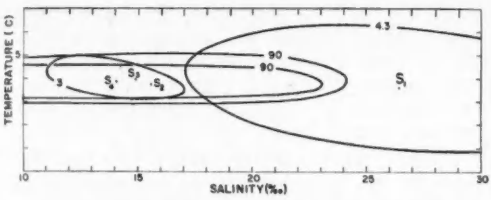

FIG. 12. Computed response centres and near-optimal isopleths for surfaces in the salinity–temperature planes from Fig. 6, 7, 9, and 11. The centers are: s_1 — larval size; s_2 — percent total hatch; s_3 — percent viable hatch; s_4 — percent postmature unhatched eggs. s_1 to s_3 are maxima, s_4 is a minimum. The isopleths shown are: larval size — 4.3 mm, total hatch — 90% (outer), viable hatch — 90% (inner), postmature unhatched — 3%. Calculated optimum and near-optimum responses occur within a restricted temperature interval but over a wide range of salinities.

mum response exclude higher salinities within the natural spawning range (suggested as 30‰ or greater). The highest salinity tested was 27.29‰, as earlier studies indicated that optimal salinity for egg development lay in the vicinity of 19‰ (Forrester and Alderdice 1966). In this context it is noted that survival of Pacific cod *larvae* is improved when larvae are held in salinities lower than that of full sea water. Conditions for optimum survival of larvae have been reported as 22‰ S and 7–8 C (Yamamoto and Nishioka 1952).

Other physical factors present in the natural environment but absent in the current tests could influence the responses measured. Salinity of the spawning medium and hydrostatic pressure are two variables not considered. Kinne (1962) has shown that subsequent development of teleost eggs is altered by the salinity of the spawning medium. The effects of hydrostatic pressure on egg development at various depths is not at all well understood.

The circumstances found experimentally to favour development of cod eggs may be compared with those under which the eggs normally are laid and incubated. Pacific cod spawn at or near the end of the period of winter cooling and larval production coincides with the start of the summer heating cycle. In northern Asian waters spawning cod frequent areas with temperatures between 0 and 2–3 C. In waters of southwestern Sakhalin and Peter the Great Bay, spawning occurs in areas with temperatures of 2–3 C. In the littoral areas of the southeastern coast of Korea, temperatures in the spawning season are 5 C or less (Moiseev 1953 p. 99–100). In waters of western Korea, the southern Kuril Islands and Hokkaido, cod are said to spawn at temperatures of 5–9 C or less. In Canadian waters, temperatures at spawning depths are approximately 6–7 C on the northern part of the coast, and 7–9 C in the southern region (Ketchen 1961).

Based on the experimental evidence, temperatures of 3.5–4 C appear optimal for Pacific cod egg development, and appreciable egg survival (50% or greater) would be expected within a temperature range from 2.5 to 8.5 C. It would thus appear that bathymetric distribution of cod in the spawning season is associated with an avoidance of supra-optimal and infraoptimal spawning temperature — the latter in northern Asian waters, and the former in southern parts of the Asiatic distribution as well as in Canadian waters.

GEOGRAPHIC DISTRIBUTION

Adult Pacific cod appear to tolerate both higher and lower temperatures than are required for reasonable survival and development of cod eggs. The limited extent of cod migrations presumably would indicate that the distribution of adult cod populations and of their spawning areas are reasonably congruent in regions bordering their northern and southern limits of geographic distribution. Therefore, conditions that may limit geographic distribution, including temperature in particular, are presumed to be those associated with a reasonable potential for egg survival and development.

Low temperature

The lowest temperature examined in the present tests (trial 12, 2 C) produced total hatches and viable hatches of 5.03 and 1.95%, respectively. Temperatures estimated to provide 50% total and viable hatches are about 2.3 and 2.5 C, respectively (Fig. 7*a*, 9*a*). Yamamoto (1939) incubated eggs at 1.2 C (Korean cod) and achieved total and viable hatches of 52.8 and 9.4%, respectively (at salinities of 33.2–34.4‰). At an incubation temperature of 2.7 C these values rose to 54.1 and 12.2%. Yamamoto and Nishioka (1952) obtained no larvae at an incubation temperature of 1 C.

Spawning cod apparently avoid temperatures below 0 C (Moiseev 1953). Based on the available evidence total hatches of 50% are associated with a temperature between 1.2 and 2.7 C, whereas 50% viable hatch is estimated to occur at an incubation temperature of about 2.5–2.7 C. The marked changes in egg survival related to incubation temperatures of the above order suggest that spawning areas with this temperature range could suffer

major fluctuations in larval production as a result of minor annual differences in temperature. It is concluded that the availability of environmental temperatures of 2.5–2.7 C in the spawning period may, to a major extent, determine the northern limits of distribution of the Pacific cod.

In the Bering Sea there is a marked decline in abundance of cod northward to Bering Strait, though they have been observed in abundance in some regions at certain times of the year (Andriashev 1937; Alverson MS 1967). No Pacific cod have been reported north of Bering Strait (Alverson MS 1967; Pruter and Alverson 1962). Waters of the northern and eastern Bering Sea and southeastern Chukchi Sea overlying the continental shelf are shallow and subject to marked seasonal temperature changes. Ellson et al. (1950) recorded bottom temperatures from less than 0 to over 6 C in the northern Bering Sea (June–July 1949), and best cod catches were associated with bottom temperatures between 2 and 4 C. Bottom temperatures in the southeastern Chukchi Sea in winter are said to approach freezing (−1.6 C) (Pruter and Alverson 1962). Dodimead (1961) indicates that winter bottom temperatures over the major portion of the continental shelf in the Bering Sea are less than 3.5 C; furthermore, over much of this area bottom temperatures are probably less than 2 C. In the southeastern Bering Sea bottom temperatures in winter probably do not exceed 2 C (Dodimead et al. 1963). The experimental evidence of temperature effects on egg development suggest that Pacific cod in the northern, eastern, and southeastern regions of the Bering Sea constitute transient, feeding populations that presumably spawn in deeper warmer water south or west of the continental shelf. It is inferred from the data that distribution on the continental slope is restricted to the limited distances travelled by cod in their feeding excursions, and by the lack of suitable spawning temperatures, 2.5 C or higher, over most of the Bering Sea flats regions during the winter spawning season.

High temperature

The highest temperature examined in the present tests was 10 C (trial 13; 20‰ S, 6.10 ppm O_2), yielding a total hatch and viable hatch of 40.29 and 37.67% respectively. Only one out of the four tests at 9.3 C (trials 3, 4, 7, 8) produced total or viable hatches in excess of 50%. On the basis of the experimental evidence (Fig. 7, 9), supra-optimal temperatures associated with total and viable hatches of 50% are those, respectively, between 8–9 and 7–8 C. Examination of the data of Yamamoto (1939 tables 2A, 2B) suggests that total hatch drops below 50% at temperatures above 8–9 C. Data of Yamamoto and Nishioka (1952) (see Forrester 1964 fig. 5) suggest that total hatch for their material would drop below 50% at temperatures above 7–8 C.

Moiseev (1953) indicates that Pacific cod will move into a spawning area when temperatures are decreasing but will not spawn at temperatures above 10 C. Cod in the southern areas of Asian distribution may enter a spawning area at temperatures of 12–13 C but will delay spawning until temperatures fall to about 5–9 C or less. In coastal waters of the Sea of Japan off the Island of Honshu catches of cod diminish southward. South of Honshu (East China Sea Region) no catches of cod are recorded for the 1957–61 period (Ministry of Agriculture and Forestry 1958, 1959, 1960, 1961, 1962). Fishing gear records for the Sea of Japan indicate that the majority of cod are caught offshore in deeper water. Although cod are caught by types of gear associated with littoral areas (e.g., fixed nets) in the northern Sea of Japan, they are seldom caught by such gear to the south. Sea temperatures for the upper 100 m (February–March 1960–64) in these coastal areas averaged approximately (Maritime Safety Agency no date): Japan Sea Northern Region — 9 C (range 8–11 C); Japan Sea West Region — 11–11.5 C (range 8–14 C); East China Sea Region — no data, but temperatures higher than West Region. Exact information is lacking on location, depth, and temperatures at which spawning might occur in these areas. These data, although admittedly meager, suggest that temperatures greater than about 9 C inhibit the movement of cod into shallow coastal waters of Honshu south of the Japan Sea North Region.

Off the Canadian coast cod normally are associated with temperatures of 6–9 C, and in the spawning period are found at depths of about 100–135 m (summarized from Ketchen 1961). In these waters in late winter the upper mixed layer is estimated to extend to about 50 m, although its depth is subject to considerable regional variation (see Ketchen 1961 fig. 3–7 incl.). Nevertheless surface temperatures are reasonably indicative of temperatures at the bottom of the upper mixed layer (Dodimead 1961; Dodimead et al. 1963; Uda 1963).

In Fig. 13 the statistical fishing areas for these waters (Pacific Marine Fisheries Commission 1968) are illustrated, along with estimates of: (*a*) the southern limit of commercial fishing for cod (i.e., the limit of fishing where cod are frequently the prime target of the otter trawl fishery or comprise a substantial incidental landing, >100,000 lb

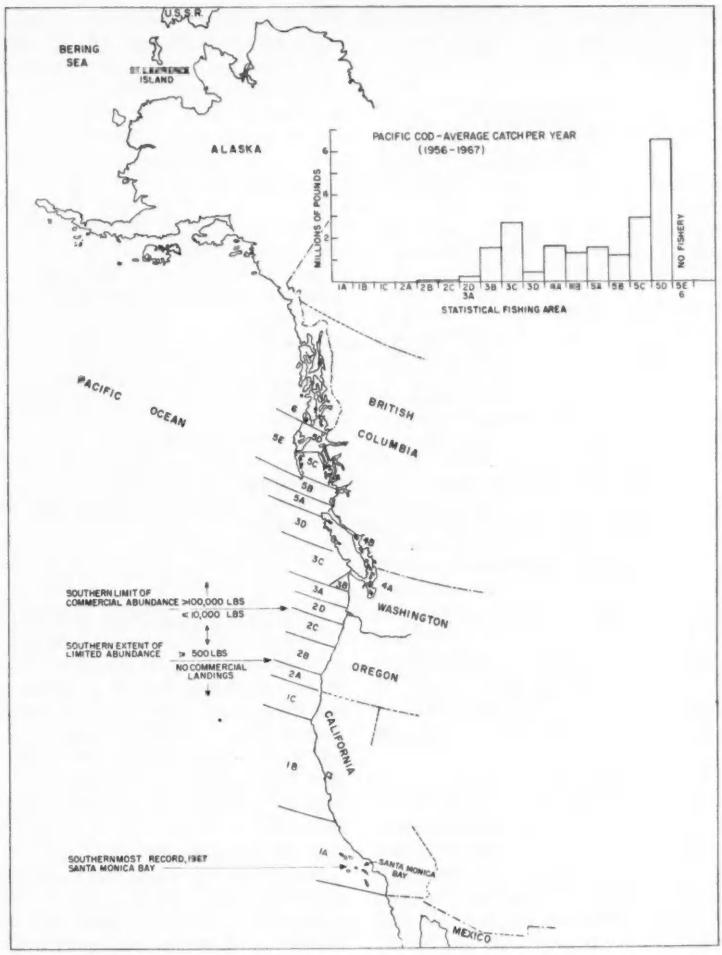

FIG. 13. Statistical fishing areas on the Pacific coast of Canada and USA in relation to distribution and commercial landings of Pacific cod.

annually); (*b*) the southern limit of the commercial fishery in which cod occur infrequently in landings of other species (total landing from 500 to 10,000 lb annually); (*c*) the southernmost record of the occurrence of Pacific cod; and (*d*) average annual landings of cod for the various statistical fishing areas. In Fig. 14 the statistical fishing areas have been indicated in relation to both sea surface temperature (Hollister MS 1964; U.S. Department of Commerce 1962) and temperature of the upper mixed layer in February (compiled from Eber et al. 1968; Dodimead 1961; International North Pacific Fisheries Commission 1961, 1964, 1967; Uda 1963), and to estimates of larval production as a function of temperature from the current study. These relations suggest that temperatures of the upper mixed layer in February are approximated by surface temperature,[6] and are associated with

[6]From Fig. 14, surface temperatures in western Alaska waters would appear to border low temperature conditions critical to egg incubation. Limited winter hydrographic records in these areas indicate that water temperatures at the bottom of the upper layer (100 m or

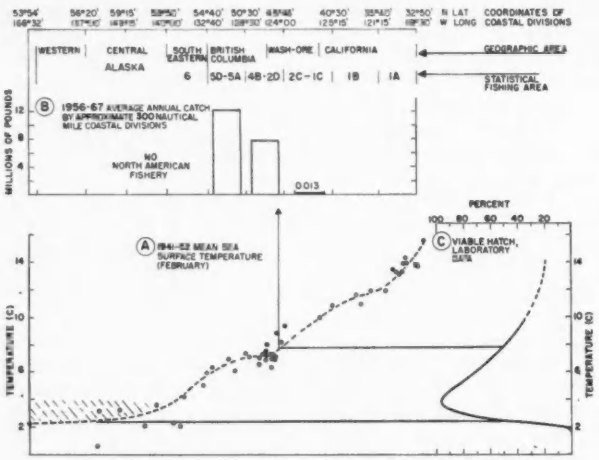

FIG. 14. Relation of (A) surface temperature (open circles) as estimates of the temperature of the upper mixed layer (solid circles), to (B) catches of cod in statistical fishing areas grouped in intervals of approximately 300 miles, and to (C) estimates of viable hatch in relation to incubation temperature from the current study.

cod distribution approximately as follows: southern limit of commercial abundance — 9 C; southern extent of limited landings — 10 C; southern limit of occurrence — 13.5 C. These estimates are in general agreement with corresponding southern limits of distribution of Pacific cod in Japanese waters.

Over the extensive geographic range of the Pacific cod genetic isolation of regional stocks may have given rise to a number of population types within the species. Moiseev (1953 p. 21) indicates the possibility of distinguishing five forms of the Pacific cod in Asian waters from morphometric data. Genetic isolation could also produce physiological differences in response to abiotic factors, such as temperature.

Although the current study employed eggs from one presumably isolated British Columbia spawning stock, developmental aspects related to temperature are in general agreement with data from the Asian extreme of the species range. Nevertheless, small physiological differences could occur among stocks that the limited available data on cod egg development would as yet be unable to detect.

By and large, it appears that the Pacific cod has rather specific temperature requirements for egg development. Its cyclic migratory behaviour appears to allow the selection of a suitable spawning environment from among a considerable array of hydrological conditions. Variations in spawning and prespawning behaviour could be construed as flexible responses of the adult to supra- and infra-optimal temperature regimes, leading in both instances to incubation temperatures within the zone of tolerance for developing eggs.

Waters of southern British Columbia and the adjoining United States coast, as well as southern parts of the Sea of Japan, are transitional for Pacific cod in terms of temperature. It is assumed that minor annual fluctuations or trends of change in coastal temperatures could be associated with fluctuations in cod abundance. Increased temperatures presumably could lead to inhibition of spawning and temperature-selective relocation of spawning adults, or to reduction of egg viability and ensuing year-class strength. The hypothesis is advanced that increases in temperature of the upper mixed layer on the southern British Columbia–Washington coast in the spawning period would lead to decreases in abundance of spawning progeny of Pacific cod entering the fishery 2–3 years later.

Historically, information has been more readily obtainable on the depths at which cod are caught commercially. As a result, emphasis has been placed

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greater) are 1 or 2 degrees C warmer than surface temperatures in Fig. 14 would indicate (Dodimead et al. 1963; Favorite 1967), consistent with the known distribution of cod in those waters (International Pacific Halibut Commission 1964; Hitz and Rathjen 1965; Alverson MS 1967).

largely on the bathymetric distribution of cod in relation to catch. On the basis of the existing evidence, it is suggested that more extensive temperature observations should be made, in association with bathymetry and measures of abundance, in order to arrive at a better understanding of the dynamics of Pacific cod populations.

Acknowledgments

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Habitat Segregation in Sanguinicolid Blood Flukes (Digenea) of Scorpaenid Rockfishes (Perciformes) on the Pacific Coast of North America

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HOLMES, J. C. 1971. Habitat segregation in sanguinicolid blood flukes (Digenea) of scorpaenid rockfishes (Perciformes) on the Pacific coast of North America. J. Fish. Res. Bd. Canada 28: 903–909.

Aporocotyle macfarlani Holmes, 1971, was found in five species of *Sebastes* taken from the inland waters around the San Juan Islands, Washington, or the inshore waters around Vancouver Island, B.C. *Psettarium sebastodorum* Holmes, 1971, was found in 14 species of *Sebastes* from the same locations or from deeper offshore waters west of Vancouver Island. The two flukes have different dominant hosts. Their numbers show different relations with depth. *Aporocotyle macfarlani* appears to be associated with fish that frequent reefs.

Aporocotyle macfarlani occupies the lumen of the afferent branchial arteries, the ventral aorta, and rarely parts of the heart; *P. sebastodorum* is threaded into the intertrabecular spaces of the ventricle and atrium. The overlap in the distributions of the two species is reduced in concurrent infections.

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AN increasing number of studies have shown that parasites have distinct recognizable microhabitats within their vertebrate hosts, although temporarily they may migrate away from those microhabitats (Read and Kilejian 1969; Cannon and Mettrick 1970; Chappell et al. 1970; Hopkins 1970). Taxonomically (or functionally) related parasites within the same species of host tend to occupy distinctly different microhabitats (Holmes 1961; Schad 1963; Martin 1969; Stockdale 1970). Microhabitat segregation has been particularly well demonstrated with gastrointestinal helminths of fishes (Sogandares-Bernal 1959; Chappell 1969; MacKenzie and Gibson 1970; Williams et al. 1970).

A difference in the microhabitats of the adult parasites is only one of the many ways in which the total habitats of related species may be segregated. Related species of trematodes of marine fishes may show very different local distribution patterns (Margolis 1963, 1965). Such patterns may be related to depth (Smith and Williams 1967; Kabata 1970), to proximity to the coastline (Boyce 1969) or to bottom type (Arai 1967; MacKenzie and Gibson 1970). Differences in host specificity may also help segregate related trematodes, particularly those found in the blood or tissues of their hosts, that appear to have limited host ranges (Yamaguti 1958; Shulman 1961; Smith and Williams 1967; Smith 1967).

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The discovery of two species of blood flukes in the rockfishes (Scorpaenidae) of the Pacific coast (Holmes 1971) provided an opportunity to investigate some of the ways in which their habitats were segregated.

Materials and Methods

Rockfishes were collected from the waters around the San Juan Islands, Washington, and Vancouver Island, B.C., by angling, spearing while scuba diving, or by trawling. A few collected by angling or by trawling were kept alive in tanks of running sea water for various periods, but most of the fish were examined immediately or frozen immediately for examination later. The fish were identified with the aid of the keys and descriptions in Clemens and Wilby (1961) and Hitz (1965). In addition, frozen samples of rockfishes taken by trawling off the west coast of Vancouver Island by the research vessel *G. B. Reed* in April and May of 1968 were received from the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C. These fish had been identified on board by S. J. Westrheim. In my previous paper (Holmes 1971) the rockfishes were ascribed to the genus *Sebastodes* Gill, 1861, in accordance with general usage. In this paper they have been ascribed to *Sebastes* Cuvier, 1829, following Tsuyuki and Westrheim (1970).

As far as possible, the fork length, weight, and sex were noted for each fish. The gills were excised and each arch was removed to an individual container of tap water. The tissues along one side of each arch between the gill bar and the base of the filaments were cut and the hemibranchs peeled back to expose the branchial arteries,

which were opened and examined with a stereomicroscope for flukes. To analyze the microdistribution of the flukes, the afferent system within each gill arch was divided into six regions (Fig. 1*a–f*): the afferent trunk (*a*), the recurrent anterior afferent artery (*b*), and four arbitrary divisions of the posterior afferent artery (terminology that of Muir 1970), using natural landmarks (such as the bends in the supporting gill bars at *c*/*d* and *e*/*f*) wherever possible. The number of flukes in each region was recorded.

The heart and the isthmus were also excised and removed to individual containers. The bulbus arteriosus, ventricle, and atrium were opened and examined under tap water with a stereomicroscope, as was the ventral aorta (including the proximal portions of the afferent trunks) after its dissection from the tissues of the isthmus. The ventricle and atrium were further examined by teasing them apart to reveal flukes in the intertrabecular spaces. The number of flukes in each region was recorded. Selected pieces of host tissue containing blood flukes were fixed in Bouin's, sectioned, and stained with hematoxylin and eosin.

The data on the numbers of worms per infected fish are expressed as medians, since the distributions of those numbers were markedly skewed and most of the samples were small. Under such conditions, means may be very misleading. Differences between medians were tested for significance using the Mann-Whitney rank test (Campbell 1967). Differences in the proportions of fishes infected, or the proportions of worms in different parts of the cardiac and branchial circulation, were tested by Chi-square tests (Campbell 1967).

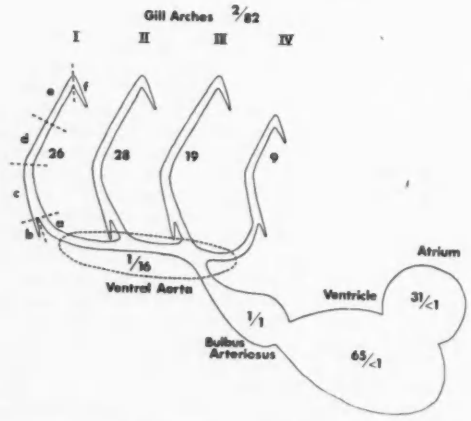

FIG. 1. Intrahostal distribution of *A. macfarlani* and *P. sebastodorum*. Figures are the percentages of the total number of worms found in each organ of the host; the numerator equals *P. sebastodorum*, the denominator *A. macfarlani*. Figures for individual gill arches are for *A. macfarlani*. Percentages are based on 1456 *A. macfarlani* and 458 *P. sebastodorum*.

Distribution among Rockfishes

Both *Aporocotyle macfarlani* Holmes, 1971, and *Psettarium sebastodorum* Holmes, 1971, were found in several species of rockfishes (Table 1). Neither was found in a thorough examination of the heart and branchial circulation of a small sample of other bottom fishes, including four *Theragra chalcogrammus* (Pallas, 1811) (whiting or Pacific pollock), two *Gadus macrocephalus* Tilesius, 1810 (Pacific cod), one *Merluccius productus* (Ayres, 1855) (Pacific hake), five *Ophiodon elongatus* Girard, 1854 (ling cod), four *Hexagrammos decagrammus* (Pallas, 1810) (kelp greenling), one *Hemilepidotus hemilepidotus* (Tilesius, 1810) (red Irish lord), one *Platichthys stellatus* (Pallas, 1811) (starry flounder), and two *Agonus acipenserinus* (Tilesius, 1811) (sturgeon poacher), all taken from the same locations as infected rockfishes. Less thorough examinations of an additional 82 specimens of 24 species belonging to 8 families of teleosts also failed to reveal either species of blood fluke.

Within the genus *Sebastes*, *A. macfarlani* appears to have a more limited host range (five species) than does *P. sebastodorum* (14 species), a limitation that may be due to ecological factors. *Aporocotyle macfarlani* was recovered from several locations in the inland waters around the San Juan Islands and from inshore waters at several locations on Vancouver Island, but not from any of the offshore locations (Fig. 2), whereas *P. sebastodorum* was recovered from all three areas. Each species was recovered from about half of the species of rockfishes examined from areas where it was present (*A. macfarlani* from 5 of 9, *P. sebastodorum* from 14 of 24 species).

One of the principal ecological factors involved appears to be the depth at which the hosts were taken, or some factor correlated with depth (Table 2). The percentage of the fish infected with *A. macfarlani* did not change significantly with depth, but the numbers per infected fish were considerably higher in the shallower waters ($P < 0.001$). In the inshore waters, a significantly higher proportion ($P < 0.001$) of the rockfish taken in the deeper water were infected with *P. sebastodorum* and the numbers of that fluke were significantly greater than in rockfish taken in shallower waters ($P < 0.001$). No such relation with depth was found in the offshore samples, but the samples from shallower depths were very small.

The different species of rockfishes harbor different numbers of blood flukes; the dominant hosts of the two species of flukes are different. The data on rockfishes from the inshore waters, where the samples were larger, show these factors best (Table

TABLE 1. Prevalence and intensity of infection with blood flukes in rockfishes.

	Inshore waters					Offshore waters		
		A. macfarlani		*P. sebastodorum*			*P. sebastodorum*	
	No. examined	No. (%) infected	Median (range)	No. (%) infected	Median (range)	No. examined	No. (%) infected	Median (range)
Sebastes								
aleutianus						3		
alutus						9		
aurora						7	3 (43)	4(2–13)
brevispinus						5	1[a](20)	22
caurinus	63	55(87)	22(1–184)	18(29)	2(1–24)			
crameri						6	4 (67)	7.5(2–8)
diploproa						5	2 (40)	1
elongatus	1					6		
flavidus	5	1(20)	1	2(40)	1	3	2 (67)	4(1–7)
helvomaculatus						6	1 (17)	1
maliger	42	27(64)	6(1–74)	25(60)	6(1–36)			
melanops	21	5(24)	2(1–3)	2(10)	1			
miniatus	3							
nigrocinctus	8			1(13)	1			
paucispinus	1					5	4[a](80)	2(1–5)
pinniger	11	2(18)	1	5(45)	2(1–5)	3		
proriger						7	2[a](29)	1
reedi						3		
ruberrimus						3		
rubrivinctus						5		
saxicola						6		
wilsoni						3		
zacentrus						6	5 (83)	5(1–16)
Sebastolobus								
alascanus						3		

[a]New host record.

1). *Sebastes caurinus* (Richardson, 1844) was more frequently infected with *A. macfarlani*, and harbored significantly more worms per fish, than any of the other rockfishes. *Sebastes maliger* (Jordan and Gilbert, 1880) also harbored substantial numbers of *A. macfarlani*; other rockfishes were less frequently infected, usually with only one or two worms. In inshore waters, *S. maliger* was the dominant host for *P. sebastodorum*, followed by *S. pinniger* (Gill, 1864) and *S. caurinus*.

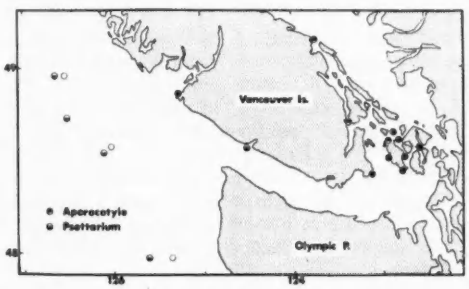

FIG. 2. Geographical distribution of *Aporocotyle macfarlani* and *Psettarium sebastodorum*.

Distribution within Hosts

Individuals of both *S. caurinus* and *S. maliger* frequently harbored both *A. macfarlani* and *P. sebastodorum*; however, the two species of flukes were not usually found in the same part of the vascular system.

Aporocotyle macfarlani was always found in the lumen, usually in the afferent branchial arteries, less frequently in the ventral aorta, and rarely in the bulbus arteriosus or other parts of the heart (Fig. 1). Only rarely did they project, even partially, into the afferent artery to the gill filaments. Their

TABLE 2. Prevalence and intensity of infection with blood flukes in relation to depth.

		Inshore waters				Offshore waters		
		A. macfarlani		*P. sebastodorum*			*P. sebastodorum*	
Depth (*fath*)	No. examined	No. (%) infected	Median (range)	No. (%) infected	Median (range)	No. examined	No. (%) infected	Median (range)
0–50	126	72(57)	9(1–184)	37(29)	2(1–24)	2	1(50)	7
51–100	17	9(53)	3(1–6)	13(76)	10(1–36)	13	3(23)	1
101–200						67	15(22)	3(1–22)
201+						12	5(42)	4(2–13)

anterior ends were directed toward the heart; they appeared to be held in place by wedging their lateral cuticular spines into the wall of the blood vessel (Fig. 3).

In general, specimens of *A. macfarlani* tended to be concentrated in the afferent trunk and in those parts of the posterior afferent branchial artery near the middle of the gill arch (regions *a*, *c*, and *d*, Table 3). The distribution did vary significantly ($P < 0.001$) among the four arches (Table 3). To test the effect of the number of worms present, the distribution of the worms in infections of 31 or more worms was compared with that in infections with fewer worms; the distributions were significantly different ($P < 0.001$), with a higher proportion of the worms located in the more proximal regions (*a*, *b*, and *c*) in the heavier infections. To test the effects of the size of the host, the distribution in *S. caurinus* over 30 cm in length was compared with that in smaller *S. caurinus*; the distributions were different ($P < 0.001$), with a higher proportion of the worms in the more distal regions (*e* and *f*) of the larger fish. The *A. macfarlani* were also located more distally in *S. maliger* than in *S. caurinus* ($P < 0.001$). The data for these comparisons are available from the author.

Psettarium sebastodorum was found primarily in the intertrabecular spaces of the ventricle (Fig. 3) or the atrium (Fig. 4). The few specimens found in the ventral aorta or branchial arteries appeared to be dead and degenerating. In infections with 11 or more *P. sebastodorum*, a significantly larger proportion (45%, as compared with 19%; $P < 0.001$) were found in the atrium. There were no significant differences in the distributions of the worms in fish of different sizes; nor was there a difference between the distributions in *S. caurinus* and *S. maliger*.

Periodic checks were made of the other major blood vessels, but neither species of fluke was ever found outside the heart and afferent branchial circulation.

TABLE 3. Distribution of *Aporocotyle macfarlani* in the afferent branchial arteries within the gill arches. The regions are those shown in Fig. 1. Values given are the numbers of worms in each region, and, in parentheses, the percentages of the total number of worms in that gill arch.

	Gill arch				
Region	I	II	III	IV	Total
a	25(12)	66(31)	53(33)	19(31)	163(25)
b	8(4)	9(4)	8(5)	0	25(4)
c	59(28)	49(23)	39(25)	24(39)	171(27)
d	60(29)	57(27)	23(14)	11(18)	151(23)
e	46(22)	26(12)	29(18)	8(13)	109(17)
f	10(5)	7(3)	7(4)	0	24(4)
Total	208	214	159	62	643

Concurrent Infections

In the rockfish taken from inshore waters, concurrent infections with both species of blood flukes occurred somewhat more frequently than expected by chance, but the difference was not statistically significant ($P > 0.10$). All of the *S. caurinus* infected with *P. sebastodorum* were also infected with *A. macfarlani*; significantly more *A. macfarlani* were found in rockfish concurrently infected with *P. sebastodorum* (medians: 39 and 17.5; $P < 0.001$) than in those without *P. sebastodorum*. In *S. maliger*, the median numbers of *A. macfarlani* (eight in single-species infections, five in concurrent infections) and *P. sebastodorum* (four and one-half and six) were not significantly different.

The distributions of the two flukes were altered in concurrent infections. *Psettarium sebastodorum* showed the more marked change, a highly sig-

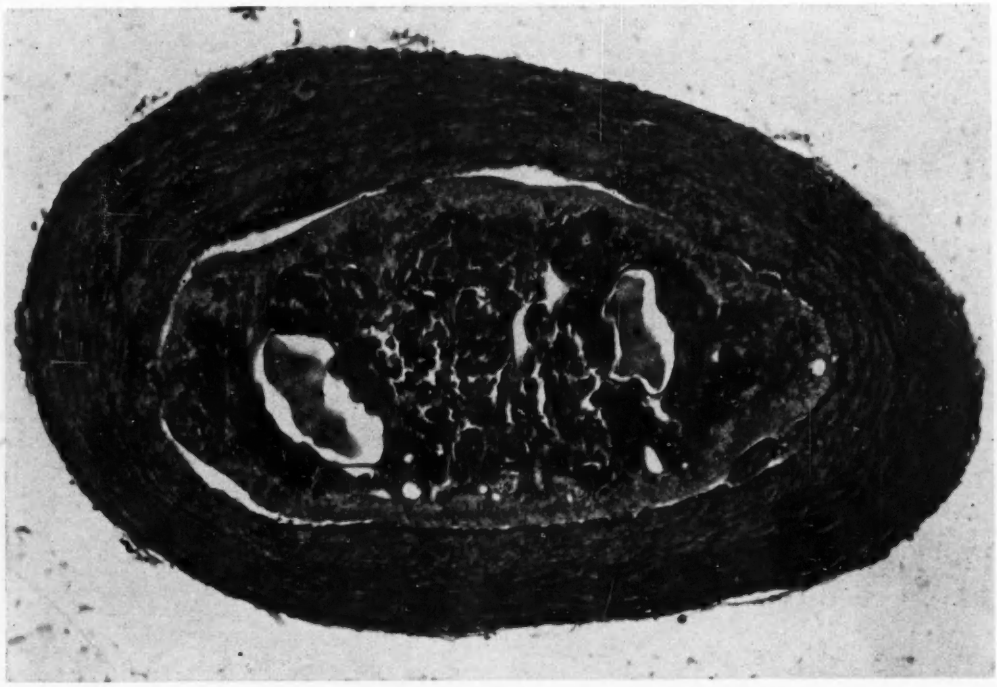

FIG. 3. Section of the ventral aorta of *Sebastes caurinus* showing *A. macfarlani* in situ.

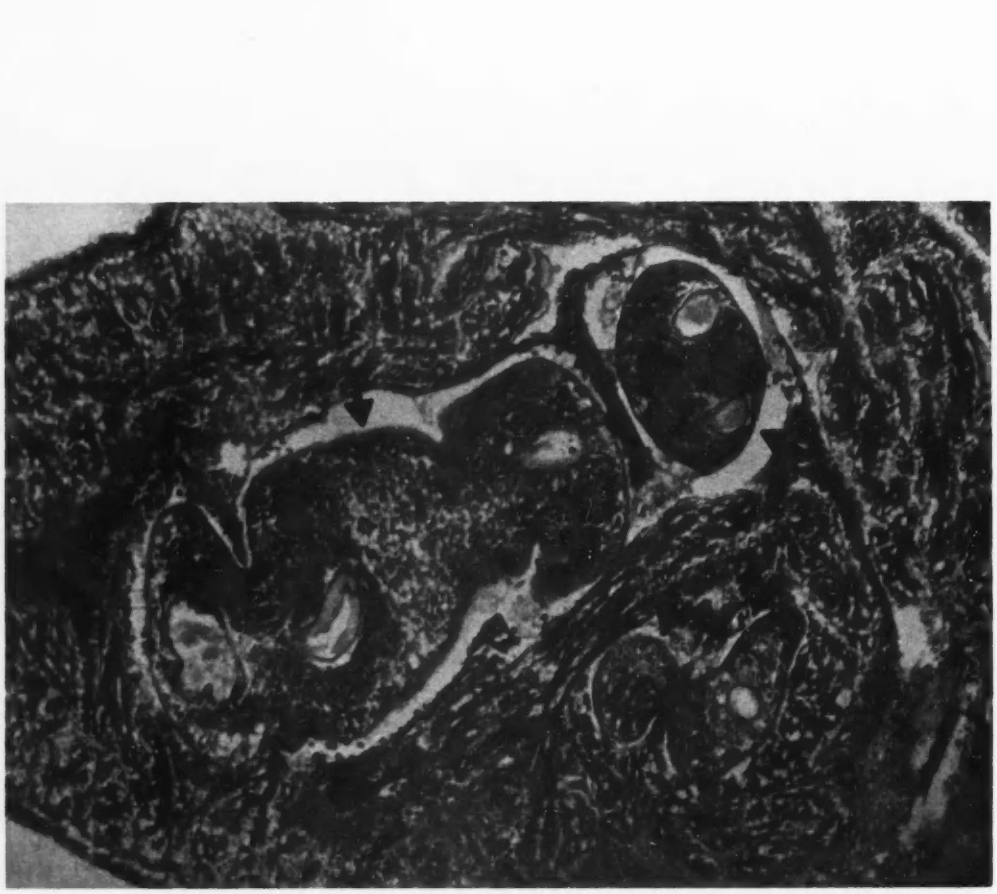

FIG. 4. *Psettarium sebastodorum* in situ in the atrium of *Sebastes caurinus*. Arrows indicate sections of the worm.

nificant ($P < 0.001$) increase in the proportion of worms in the atrium and a decrease in the proportion in the ventricle (Table 4). The change in the distribution of the *A. macfarlani* was less dramatic, but was also significant ($P < 0.05$); fewer worms were found in the ventral aorta, more in the afferent branchial arteries in the gills. The net result of these changes was a reduction in the amount of overlap in the distribution of the two species in concurrent infections.

Discussion

Relatively little critical work has been done on the host specificity of marine sanguinicolids. *Aporocotyle simplex* Odhner, 1900, has been reported from three genera of the Pleuronectidae, plus *Sebastes* (Scorpaenidae) and *Theragra* (Gadidae). However, the records from *Sebastes* refer to *A. macfarlani* (see Holmes 1971) and those from *Theragra* probably refer to *A. theragrae* Ichihara, 1970 (see Smith 1967; Ichihara 1970). Smith and Williams (1967) found *Aporocotyle spinosicanalis* Williams, 1958, in hake (Merluccidae) but not in 268 other gadoid fish belonging to four species. These observations, plus those made in this study, suggest that specificity to at least the family level may be characteristic of species of *Aporocotyle*. *Psettarium sebastodorum* also appears to be specific to the genus *Sebastes* (or to the family Scorpaenidae); little information is available for the other two species of this genus: *P. japonicum* (Goto and Ozaki, 1929) has been reported three times, from two species of *Sphaeroides* (Tetraodontidae), and *P. tropicum* Manter, 1940, once, from *Cheilichthys annulatus* (Tetraodontidae).

Sanguinicolid trematodes lack any vestiges of oral or ventral suckers. They are able to maintain their positions in their hosts by means of well-developed tegumental spines or by selecting relatively quiet habitats. *Aporocotyle macfarlani* and *P. sebastodorum* have adapted to different habitats by adopting dissimilar means of attachment. *Aporocotyle macfarlani* is a relatively short, fairly stout-bodied fluke armed with patches of tegumental spines along the lateral margins of the body. Their anterior ends are directed into the current, and they apparently drift backwards with the current until they reach vessels small enough that their tegumental spines wedge into the walls of the blood vessels. In the larger blood vessels of large *S. caurinus* (or of *S. maliger*, which has larger branchial vessels than *S. caurinus* of comparable length), *A. macfarlani* tends to drift into the more distal vessels. When large numbers of flukes are present, groups of them collectively may wedge into larger, more proximal vessels.

The geometry of the ventral aorta and its branches and the relative flow of blood through them appear to determine the distribution of *A. macfarlani* among the gill arches. There is a relatively sharp backward bend into arch IV (and into the anterior afferent

TABLE 4. Distribution of blood flukes in single species and concurrent infections.

	Single		Concurrent	
	Number	% of total	Number	% of total
Aporocotyle macfarlani				
Heart	19	2	7	2
Ventral aorta	146	17	85	13
Gill vessels (total)	649	77	550	83
Arch I	196	23	189	29
II	231	27	181	27
III	160	19	117	18
IV	62	7	63	10
Wash	28	3	21	3
Total	842		663	
Psettarium sebastodorum				
Atrium	36	17	104	41
Ventricle	160	77	136	54
Bulbus arteriosus	1	<1	5	2
Other locations	10	5	6	2
Total	207		251	

artery, region *b*). These areas also appear to have less flow through them than do the other regions or the other gill arches; they are seldom occupied by *A. macfarlani*.

Psettarium sebastodorum is a longer narrower fluke with a fairly large, asymmetrical posterior lobe and combs of spines along the lateral margin. The worms are usually threaded into the intertrabecular spaces, where they are held in place by loops of the body, aided by the tegumental spines. The posterior lobe, in which are located the genital pores, usually protrudes into the main cavity of the ventricle or atrium, and may act in part as an anchor to prevent the worm from slipping out anteriorly. *Psettarium sebastodorum* appears to be well adapted to a substrate with relatively narrow, interconnected passages, like the atrium, ventricle, or, to a lesser extent, the proximal part of the bulbus arteriosus.

The life cycles of marine sanguinicolids are largely unknown. Several cercariae have been allocated tentatively to this family (or to the Aporocotylidae, a synonym) on the basis of the absence of suckers, the presence of an X- or H-shaped intestine, or the presence of lateral combs of tegumental spines. These cercariae are produced by sporocysts (or rediae) in pelecypods (Linton 1915; Martin 1944; Holliman 1961) or tubicolous polychaetes (Stunkard 1929; Martin 1952; Oglesby 1961). Both groups of potential intermediate hosts are well-represented in the localities from which infected fish have been taken.

Several of the correlations found in this study suggest that the intermediate host of *A. macfarlani* lives on shallow reefs or rock walls and that of *P. sebastodorum* is either ubiquitous in this part of the continental shelf, or lives on the bottom away from reefs. Most of the fish taken in the shallow inshore waters, where *A. macfarlani* was common, but *P. sebastodorum* was not, were taken by angling or spearing from reefs or rock walls. All of the fish taken in the offshore waters, and those taken in the deeper inland waters, where *P. sebastodorum* was common but *A. macfarlani* was absent or less common, were taken by trawling from areas without reefs.

The predominant host of *A. macfarlani*, *S. caurinus*, appears to be much more closely bound to reefs than are *S. maliger*, *S. melanops*, or other rockfishes less frequently infected with this fluke. General observations made while diving indicated that *S. caurinus* was the most abundant of the rockfishes on the reefs, was rarely encountered on the level bottom away from the reefs, and apparently spends most of its time resting directly on the reef, making short dashes along the reef or into the water above the reef to catch food.

The predominant host of *P. sebastodorum* in inshore waters, *S. maliger*, was often encountered on reefs, but more frequently was seen on level bottoms near reefs. These rockfish also spent most of their time on the bottom. The other common inshore rockfish, *S. melanops*, appeared to be most frequent along rock walls, swimming actively in schools a short distance away from the wall. They were rarely found resting on the bottom. Unfortunately, almost nothing is known about the details of the habitats of the deeper water rockfishes harboring substantial numbers of *P. sebastodorum*.

Aporocotyle macfarlani appears to have a more restricted geographical range than *P. sebastodorum*. In addition, their habitats appear to be segregated by having different dominant hosts, different depth relations (both of which may be due to different relations with reefs), and different microhabitats within the cardiac-branchial circulation. The last difference is intensified in concurrent infections.

Acknowledgments

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Particulate Carbon:Nitrogen Relations in Northern Chesapeake Bay[1]

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FLEMER, D. A., AND R. B. BIGGS. 1971. Particulate carbon:nitrogen relations in northern Chesapeake Bay. J. Fish. Res. Bd. Canada 28: 911–918.

The Susquehanna River annually supplies about 8.4×10^4 and 4.7×10^3 metric tons of particulate carbon (PC) and nitrogen (PN), respectively, to upper Chesapeake Bay. In the upper bay, the concentration of PN usually ranges between 0.10 and 0.30 mg liter^{-1} and is occasionally greater than 0.50 mg liter^{-1}. In the lower study area, the concentration of PN stabilizes near 0.10 mg liter^{-1}. Maximum values of the carbon:nitrogen (C:N) ratio (atomic basis) occurred in the upper bay, and highest values were associated with the late-winter thaw period of the Susquehanna River. C:N ratios of 20–30 were usual most of the year in the low salinity region, and often greater than 30 during times of maximum river discharge. In the lower study area, the ratio approached 15 throughout most of the year. In general, the reduction in the C:N ratios of the suspended material was reflected in the C:N ratios of the sediments.

The high C:N ratios in the upper bay and the tidal freshwater portion of the Susquehanna River indicate a high detrital content. An estimate of net primary production would extrapolate to the fixation of PN of approximately 1600 metric tons per year in the upper bay or about 34% of that supplied by upland drainage. The major source of PN in the lower study area is probably provided by photosynthetic fixation, since the physical circulation of the estuary retards a large movement of particulate material seaward.

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THIS study reports on the spatial distribution throughout the year of particulate nitrogen (PN) of the suspended matter at channel stations in the tidal freshwater area to about the midpoint on the longitudinal axis of Chesapeake Bay (Fig. 1). The seasonal pattern of input of PN by the Susquehanna River was measured to characterize the dominant terrestrial sources. Cores of the bottom sediment in the study area were analyzed for particulate carbon (PC) and PN, which permits a comparison with the suspended material. Earlier published values of PC in the suspended matter (Biggs 1970) now allow an examination of the carbon:nitrogen (C:N) ratios. An attempt is made to understand the observed pattern of the C:N ratios in terms of the physical circulation and biological processes.

A high interest in the nature and distribution of organic matter in aquatic environments is evidenced by several reviews (Hood 1970; Jørgensen 1966; Parsons 1963). Organic matter of terrestrial origin may have an important role in coastal bodies of water (Seki et al. 1969; Stephens et al. 1967; Gripenberg 1939). Chesapeake Bay, due to its relatively long salinity gradient, offers some advantages in sampling and the comparison of C:N ratios of water dominated either by terrestrial or autochthonous sources of organic matter.

Study Area

Chesapeake Bay is a partially mixed or type B estuary (Pritchard 1967). The deeper waters are more saline on the average than the surface layer. The net motion of the surface layer is seaward whereas that of the deep layer is toward the head of the estuary. The Susquehanna River contributes about 86% of the fresh water to the upper bay above the mouth of the Potomac River (Carpenter et al. 1969).

More than 90% of the area upstream from station V (Fig. 1) is less than 8 m deep. The mean depth is about 4.7 m (Shubel MS 1968, 1969). Shubel pointed out that tidal scour and wind waves in the upper study area (stations I–V) are important to resuspension of the sediment. He also described the "sediment trap" that results from the two-

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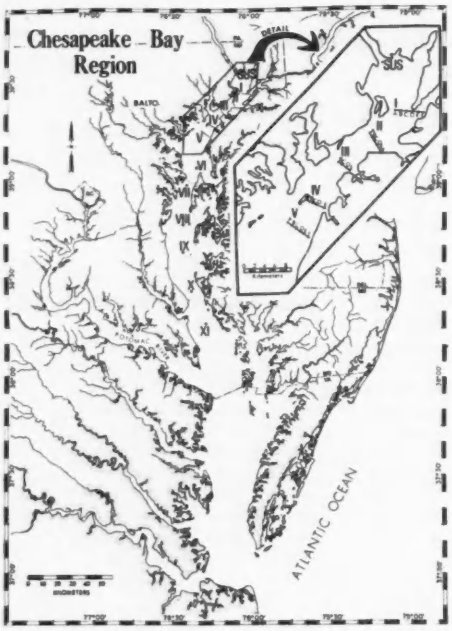

FIG. 1. Location of stations in study area.

layered circulation. The lower study area (stations VI–XI) is considerably deeper on the average, most of this region having a relatively narrow channel typically 30–40 m in depth.

The waters near the bottom of the channel during the summer sometimes become oxygen deficient (Pritchard 1952), but the effects of the relatively large volume of treated sewage discharge from the metropolitan area of Baltimore, Maryland, are not observable in the Chesapeake Bay below Baltimore (Carpenter et al. 1969).

Methods

Data on particulate carbon were taken from Biggs (MS 1969, 1970). The Dumas method of high temperature oxidation was used to determine particulate nitrogen. Analyses were carried out on a Coleman Model 29A Nitrogen Analyzer equipped with a Model 29 combustion tube and syringe. Particulate material was concentrated on Whatman GF/C filters. These filters were desiccated and frozen until analyzed. Usually, one filter was cut in half and the carbon and nitrogen analyses were run on respective sections. Data given in this paper represent approximately monthly samples obtained from November 1965 to November 1966.

Samples usually were taken at 0.5 m (surface) and every 3 m to the 12-m depth from all stations except stations II-D, III-C, and IV-D where the 9-m depth was the maximum depth sampled. In addition, samples were routinely obtained at 15 and 23 m from stations VII, VIII, IX, X, and XI and occasionally samples were taken at 31 m at these stations.

Results

Suspended Material

Particulate nitrogen

The concentration of PN showed a strong gradient over the study area (Table 1). Maximum values were found between stations I-E and VI. In this area, the mean concentration in the water column often ranged between 0.10 and 0.30 mg N liter^{-1}. Values greater than 0.30 mg N liter^{-1} occurred during the fall and winter. Seaward from station VI the average concentration seldom exceeded 0.10 mg N liter^{-1}; however, fewer data are available for the lower study area. Vertical uniformity of the concentration of PN at all stations is shown by the general similarity of the surface and bottom concentrations to the average for the water column.

An estimate of the time-weighted annual contribution of PN by the river amounted to about 4700 metric tons from November 1965 to November 1966 (Fig. 2). Much of the material accumulated during the winter thaw period, and about 55% was transported to the upper bay by the river during February. By the middle of June, 95% of the total annual contribution by the river was complete.

Carbon:nitrogen ratios

Average values of the C:N (atomic) ratios for the water column were high in the upper study area much of the year (Table 2). Values frequently ranged between 20 and 30 in this area, and during the winter and spring values were greater than 30 at station SUS. These high ratios coincide with maximum discharge from the Susquehanna River. Seaward from station VII, the ratios usually averaged less than 20 in the water column. In July, August, and September, the ratio was consistently less than 20 throughout the study area.

Sediments

PN in the sediment cores, from the bottom surface and every 5 cm down to the 30-cm depth, ranged from 0.07 to 0.31% (Table 3). Values at station VI were consistently lower than at other

TABLE 1. Distribution of particulate nitrogen (mg N liter^{-1} $\times$ 10^{-2}) in the suspended material for the northern Chesapeake Bay. Concentrations are given for the surface (S), bottom of the sampled column of water (B), and average ($\bar{x}$) for the entire water column.

	SUS	I-E	II-D	III-C	IV-D	V-F	VI	VII	VIII	IX	X	XI
						Nov. 10, 1965						
S	4.4	9.1	14.8	5.1	8.9	13.1	10.2	4.1	4.4	6.1	6.9	5.8
$\bar{x}$	6.9	11.1	12.7	6.5	9.4	7.2	12.0	3.9	3.4	6.3	–	3.9
B	8.9	14.7	9.4	7.3	11.8	5.3	11.3	3.4	2.7	6.3	3.6	3.1
						Dec. 14, 1965						
S	5.8	6.2	9.7	–	4.9	5.3	5.3	–	1.3	2.7	3.9	1.6
$\bar{x}$	5.1	7.3	9.4	4.9	8.8	3.7	4.5	1.6	2.4	3.7	3.2	–
B	4.0	7.3	7.7	3.1	15.7	5.8	5.8	1.7	0.9	2.3	1.2	–
						Jan. 20, 1966						
S		12.8	10.3	5.7	3.1	6.4	3.4	4.4	5.0	4.8(3)[a]	2.2	6.5
$\bar{x}$	Ice	11.3	9.4	6.2	4.8	5.9	4.7	4.6	4.5	6.1	6.2	5.8
B		16.4	10.0	7.3	7.3	5.5	9.2	5.7	8.1	6.5	5.4	6.1
						Feb. 15, 1966						
S	12.0	14.8	33.7	33.0	16.8	14.4	4.0	0.9	3.0	0.7	3.8	3.2
$\bar{x}$	71.4	9.4	55.7	53.1	35.5	17.6	3.5	4.5	4.8	4.9	6.3	6.8
B	61.3	9.3	82.9	51.3	34.4	7.6	1.1	7.7	6.5	4.3	6.9	9.7
						Mar. 15, 1966						
S	5.5	15.2	12.0	18.0	8.8	10.8	5.7	7.4	9.7	7.3	4.1	6.2
$\bar{x}$	5.8	18.5	13.2	15.4	12.1	12.8	5.5	5.9	9.2	10.0	6.1	6.4
B	6.1	16.0	12.0	20.0	19.3	29.0	8.9	5.8	7.5	16.7(15)	7.7	5.6
						Apr. 26, 1966						
S	10.5	68.8	12.5	10.4	7.6	2.1	4.0	5.3	3.9	7.4	6.4	5.1
$\bar{x}$	20.7	54.7	28.2	27.9	10.6	3.9	5.2	4.7	4.2	6.8	5.0	6.8
B	16.8	50.4	61.3	56.0	5.7	0.8	7.1	4.6	5.6	5.7	3.1	8.1
						May 11, 1966						
S	13.2	16.8	–	3.5	6.8	7.2	8.0	7.3	5.2	5.1	5.6	6.0
$\bar{x}$	16.7	11.8	–	3.8	7.3	7.8	5.5	7.4	6.6	6.3	6.8	7.1
B	16.5	9.6	–	3.2	12.0	8.4	3.0	4.6	8.4	9.6	10.5	11.1
						July 18, 1966						
S	20.3	18.3	22.7	25.1	24.0	23.6	13.6	19.2	–	–	–	20.8
$\bar{x}$	14.1	21.3	19.3	27.6	20.0	26.6	10.8	11.0	–	–	–	10.4
B	5.6	32.0	19.5	42.4	15.5	45.6	10.0	3.2	–	–	–	1.6
						Aug. 29, 1966						
S	10.9	44.0	20.4	41.0	27.0	17.2	11.8	–	–	8.5	–	12.0
$\bar{x}$	14.9	39.8	26.7	29.4	21.6	16.9	12.5	–	–	6.2	–	8.2
B	20.6	35.2	29.2	28.0	20.0	24.0	6.3	–	–	4.8	–	2.7
						Sept. 14, 1966						
S	18.9	31.6(3)	–	31.2	37.2	24.3	10.5	–	–	11.2	–	–
$\bar{x}$	22.2	30.6	38.4(3,6)	32.8	34.3	13.9	9.6	–	–	7.4	–	–
B	26.0	32.2	–	41.0	20.8	7.7	10.4	–	–	3.3	–	–
						Oct. 10, 1966						
S	17.8	–	22.4	20.0	–	29.2	21.6	–	–	–	–	–
$\bar{x}$	11.9	–	38.8	18.0	–	15.5	23.8	–	–	–	–	–
B	10.1	–	92.0	22.4	–	13.5	25.6	–	–	–	–	–
						Nov. 8, 1966						
S	20.0	14.2	30.7	19.6	12.8	13.8	7.9	–	–	7.3	–	11.2
$\bar{x}$	8.3	17.9	22.0	20.6	32.0	8.6	9.4	–	–	6.2	–	8.8
B	2.2	24.7	20.9	24.7	39.0	5.5	20.3	–	–	24.8	–	6.9

[a]Numbers in parentheses refer to specific depth (m) if sample was not available from the surface or bottom.

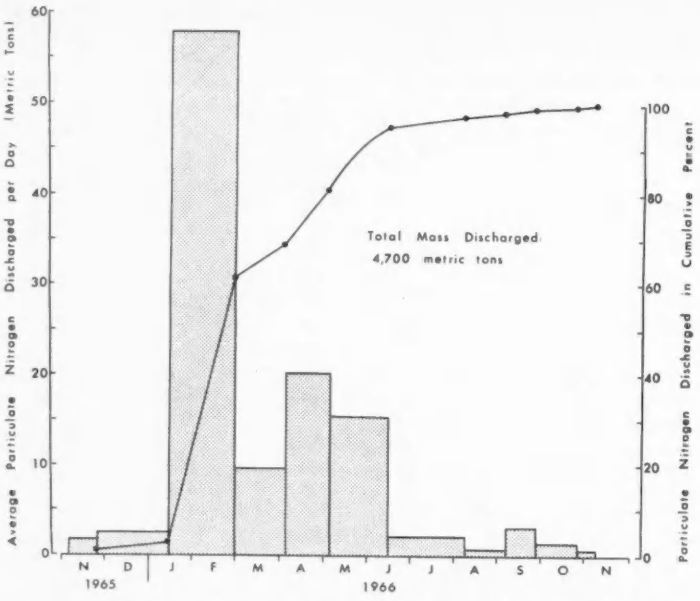
Average Particulate Nitrogen Discharged per Day (Metric Tons)
Particulate Nitrogen Discharged in Cumulative Percent
Total Mass Discharged:
4,700 metric tons
N D J F M A M J J A S O N
1965
1966

FIG. 2. Time-weighted cumulation of total particulate-organic nitrogen discharged from the Susquehanna River at station SUS during the period November 1965 to November 1966.

stations. Changes in the percent nitrogen with core depth were quite variable at individual stations; however, more low values were typical of the 29–30-cm depth. The average percent nitrogen from cores taken in the upper study area was slightly less than samples obtained from the lower study area.

PC in the sediments ranged from 0.88 to 12.86% at depths similar to those used in the nitrogen determinations. The percent PC with depth was variable and there were only slightly lower values near the bottom of cores. Due to the dredge and spoil activity in the upper bay, especially from station VI towards the head of the bay, the sediments may have been disturbed. Percent carbon in the surface sediments at stations IX and XI was similar to that found opposite the mouth of the Patuxent River (Biggs 1967).

The average C:N ratios of all depths sampled in the cores were as follows: I-C 44, II-D 29, V-F 61, VI 16, VII 18, IX 17, and XI 13. The C:N ratio decreased seaward both in the sediments and suspended material.

Data on the concentration of PN in the sediments from other areas of the Chesapeake system approximate those of the present study. Nitrogen values of surface sediments (Brehmer 1967) ranged from about 0.04 to 0.26% in the James River. In the lower Chesapeake Bay, nitrogen values of surface sediments ranged from 0.01 to 0.20% (Young 1968), and C:N ratios for the surface and 10-cm depths were 8.2 and 10.6, respectively.

Discussion

The present data on PN help to further characterize the nature and distribution of the organic matter discussed by Biggs (1970). The concentration of PN found in the lower study area (ca. 0.10 mg liter^{-1}) is typical of the limited data for coastal areas. In the upper study area, the concentration of PN, which frequently ranged between 0.10 and 0.30 mg liter^{-1}, greatly exceeds most reported values.

The measured C:N ratios were compared with those of important sources to estimate if carbon or nitrogen was mobilized preferentially in the particulate material. The assumptions are that both upland drainage and primary production account for most of the PC and PN for the upper study area, whereas primary production is the major source of these materials in the lower study area. We assume that heterotrophic fixation of

TABLE 2. Distribution of the carbon: nitrogen ratios (atomic) for the period November 1965 to November 1966 from stations SUS to XI. Ratios are given for the surface (S), bottom of the sampled column of water (B), and average ($\bar{x}$) for the entire water column.

	SUS	I-E	II-D	III-C	IV-D	V-F	VI	VII	VIII	IX	X	XI
						Nov. 10, 1965						
S	19	17	12	21	17	12	11	14	16	19	19	18
$\bar{x}$	15	16	18	17	17	29	9	17	23	18	–	25
B	13	17	27	16	15	42	4	21	26	17	26	30
						Dec. 14, 1965						
S	12	17	16	–	21	17	13	–	27	13	12	22
$\bar{x}$	14	16	16	31	18	33	14	29	16	12	20	–
B	18	16	21	45	16	16	14	27	15	15	39	–
						Jan. 20, 1966						
S		15	17	35	49	22	27	16	14	17(3)[a]	37	13
$\bar{x}$	Ice	18	19	43	35	25	24	18	18	16	15	15
B		15	17	32	26	28	23	19	12	18	13	17
						Feb. 15, 1966						
S	22	22	12	27	49	15	35	91	16	50	12	15
$\bar{x}$	24	23	9	22	29	17	51	24	13	14	11	13
B	24	30	5	25	21	32	64	11	14	22	14	14
						Mar. 15, 1966						
S	30	31	43	26	40	24	35	24	17	22	20	13
$\bar{x}$	26	36	42	31	38	27	26	18	14	14	21	17
B	30	42	40	18	30	15	18	16	16	8(15)	21	23
						Apr. 26, 1966						
S	24	7	22	25	38	33	–	15	15	9	9	14
$\bar{x}$	14	10	18	21	38	25	–	12	15	10	10	11
B	13	12	17	28	61	40	16	15	11	14	12	9
						May 11, 1966						
S	25	25	–	86	29	26	15	22	16	18	12	12
$\bar{x}$	24	46	–	114	37	20	18	17	15	17	13	11
B	22	52	–	164	35	21	27	23	15	12	9	9
						July 18, 1966						
S	9	26	15	12	12	8	8	9	9	–	–	–
$\bar{x}$	17	20	17	14	16	10	11	17	12	–	–	–
B	35	12	20	13	18	12	14	36	16	–	–	–
						Aug. 29, 1966						
S	21	10	12	7	12	12	18	–	–	10	–	9
$\bar{x}$	16	11	12	10	20	11	11	–	–	11	–	13
B	14	13	12	10	29	11	13	–	–	6	–	22
						Sept. 14, 1966						
S	12	10(3)	–	15	14	12	23	–	–	8	–	–
$\bar{x}$	27	12	15(3,6)	18	14	18	17	–	–	16	–	–
B	12	16	–	13	26	23	13	–	–	28	–	–
						Oct. 10, 1966						
S	10	–	18	14	–	11	12	–	–	–	–	–
$\bar{x}$	13	–	24	24	–	25	10	–	–	–	–	–
B	15	–	18	24	–	28	9	–	–	–	–	–
						Nov. 8, 1966						
S	8	21	11	14	40	12	12	–	–	13		16
$\bar{x}$	36	17	15	22	35	19	14	–	–	15		14
B	64	12	15	24	34	28	21	–	–	19		14
$\bar{x}$[b]	18	20	18	24	22	19	17	–	–	13		13

[a]Numbers in parentheses indicate specific depth (m) other than that shown in the first column.
[b]Time-weighted annual average for the water column.

PC and PN is not quantitatively significant in relation to other sources.

Based on the present study and previously cited work, the Susquehanna River supplied about 84,000 and 4700 metric tons per year of PC and PN, respectively, to the upper study area. Adjustments were made in the estimate of PC to correspond to the present period of sampling for PN. Biggs (1970) extrapolated from the data on gross primary production that about 8000 metric tons of PC was converted to net primary production in the upper study area. An assumed nitrogen equivalent to this net carbon fixation would be about 1600 metric tons based on a C:N ratio of

TABLE 3. Percent of carbon and nitrogen in the sediments (dry weight) and the carbon:nitrogen ratios (weight basis at specified depths and weight and atomic basis used in calculations of mean values).

	Station						
	I-C	II-D	V-F	VI	VII	IX	XI
				0–1 cm			
C	6.0	5.1	5.9	1.7	3.4	3.1	2.8
N	0.16	0.23	0.20	0.12	0.23	0.31	0.22
C:N	37.5	22.2	29.5	14.2	14.8	10.0	12.7
				4–5 cm			
C	6.8	7.9	7.7	1.4	3.5	3.8	2.9
N	0.15	0.28	0.28	0.09	0.23	0.23	0.26
C:N	45.3	22.8	27.5	15.6	15.2	16.5	11.2
				9–10 cm			
C	7.4	5.4	8.3	1.0	3.4	3.0	2.1
N	0.18	0.14	0.10	0.07	0.18	0.25	0.24
C:N	41.1	38.6	83.0	14.3	18.9	12.0	8.8
				14–15 cm			
C	7.2	3.5	9.0	0.9	3.7	3.1	2.8
N	0.23	0.15	0.20	0.11	0.27	0.15	0.23
C:N	31.3	23.3	45.0	8.2	13.7	20.7	12.2
				19–20 cm			
C	7.0	3.1	11.2	0.9	4.4	2.9	2.3
N	0.17	0.21	0.19	0.07	0.29	0.17	0.24
C:N	41.2	14.8	58.9	12.9	15.2	17.1	9.6
				24–25 cm			
C	6.9	2.4	12.1	1.1	3.1	2.8	2.0
N	0.18	0.12	0.23	0.08	0.22	0.20	0.18
C:N	38.3	20.0	52.6	13.8	14.1	14.0	11.1
				29–30 cm			
C	2.5	2.2	12.9	1.4	1.5	3.0	2.4
N	0.08	0.07	0.19	0.08	0.09	0.25	0.18
C:N	31.2	31.4	67.9	17.5	16.7	12.0	13.3
				Mean			
C	6.2	4.2	9.6	1.2	3.3	3.1	2.4
N	0.16	0.17	0.20	0.09	0.22	0.22	0.22
C:N (wt)	37.9	24.7	52.1	13.8	15.5	14.6	11.3
(at)	44.3	28.9	61.0	16.1	18.1	17.1	13.2

5.1 by weight (Strickland 1960). The estimated annual supply of PC and PN to the upper area of study is 92,000 and 6300 metric tons, respectively. If processes act on both C and N in the same degree, then the average C:N ratio of the suspended material in the estuary should approximate the ratio in the main sources of this material. For selected stations in upper and middle Chesapeake Bay, the C:N ratios calculated from estimated yearly inputs to the estuary, compared with those in the main sources, are as follows:

	Seston	Sediments	Sources
Stations I-E, II-D, III-C, IV-D, V-F, and VI	20	43	17
Stations IX and XI	13	16	6

Since the C:N ratios of sources and estuary agree closely, we conclude that the suspended sediments in the upper bay are in an approximately steady-state balance.

A similar calculation was used to obtain some insight into the C:N relations in the lower study area. Due to the physical circulation of this two-layered estuary, there is only a small net flux of particulate material from the upper to the lower study area (Biggs 1970). The C:N ratio should be about 6.0 if most of the suspended organic material is in the living state, but the calculated C:N ratio averaged 13 for the year studied. We conclude that the suspended particulate organic material in the lower study area is suffering a relative loss of nitrogen with respect to carbon.

The bottom sediments of the upper study area are frequently resuspended through wind-wave activity (Shubel and Biggs 1969). Thus, the difference in the C:N ratios between the suspended and bottom sediment is difficult to explain, especially since little vertical difference was noted in the C:N ratios of the cores. Contributing factors may be materials from upland drainage that have high C:N ratios, e.g., forest humus, terrestrial plants, and wood fiber (Gucluer and Gross 1964), and particles of coal in sediment of the upper study area (Ryan 1953). Biological processes could account for removal of nitrogen, such as through the microorganism–snail food chain (Newell 1965), as snails are abundant in the upper area (Flemer et al. 1968); but there is little change in the ratio in sediments in the lower area, where lack of a permanent macrobenthic community may provide the explanation.

It is also of interest to consider the remineralization of the PN, which may help explain the pattern of the observed C:N ratios. The C:N ratio (13) of suspended material in the lower study area suggests that nitrogen is preferentially lost. Dissolved nitrogen is recycled into additional phytoplankton production, especially during the warm months (Carpenter et al. 1969). Primary production on a volume basis at stations I-E to III-C is about double that near Annapolis during the summer (Flemer 1970). The inorganic nitrogen concentration during the warmer months is reasonably uniform throughout the study area (Whaley et al. MS 1966). It is probable that the sediments are relatively more important as a source of nitrogen for phytoplanktonic production in the upper area than in the lower area. This is consistent with the high C:N ratio (43) of the sediments in the upper area of study and the apparent steady-state balance in the C:N ratio of the suspended material. The turbulence and small vertical density differences would aid the nutrient exchange between the sediments and overlying waters.

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NOTES

Effects of Temperature on Movements of Prespawning Brook Sticklebacks, *Culaea inconstans*, in the Roseau River, Manitoba

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MacLean, J. A., and J. H. Gee. 1971. Effects of temperature on movements of prespawning brook sticklebacks, *Culaea inconstans*, in the Roseau River, Manitoba. J. Fish. Res. Bd. Canada 28: 919–923.

During spring, brook sticklebacks (*Culaea inconstans* (Kirtland)) in the Roseau River, Man., moved upstream from deep, cool, overwintering areas to shallow warmer waters in meltwater ponds and ditches, where they reproduced. Movement upstream occurred during daylight, in water velocities less than 90 cm/sec, when a temperature gradient was present, but ceased at night or when water temperatures exceeded 19–20 C. In a horizontal laboratory gradient, prespawning sticklebacks from the field selected a range of temperatures from 14.9 to 20.2 C, considerably narrower than after spawning. More prespawning sticklebacks moved upstream in an artificial stream tank at water temperatures of 15.6 and 21.1 C than at 7.2, 10.0, or 23.9 C, and more moved upstream in light than in darkness.

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The object of this study was to determine the effects of temperature on distribution and movements during spring of prespawning brook sticklebacks, *Culaea inconstans* (Kirtland), in the Roseau River, Man.

Brook sticklebacks occur across North America, from northeastern British Columbia to New Brunswick and from Nebraska to New York, although isolated populations occur outside this range (Nelson 1969). In southern portions of its range, *Culaea* is confined to spring-fed streams and deep lakes, suggesting that water temperature may influence distribution (Winn 1960; Reisman and Cade 1967). During spring, brook sticklebacks move into shallow water, where they reproduce (Winn 1960; Reisman and Cade 1967). Data of Applegate (1961) and Lamsa (1963) suggest that water temperature and current influence this movement. Movements of brook sticklebacks in a part of the Roseau River during the springs of 1967 and 1968 are described herein, as well as laboratory tests of hypotheses to explain the movements.

Field study — The Roseau River enters the Red River 97 km south of Winnipeg. Discharge into the Red River averages from 0.2 m³/sec in winter to 28 m³/sec during spring runoff (April–June). The study area chosen was in the lower portion of the Roseau, 6.5 km upstream from the mouth. During spring, meltwater that accumulated in shallow ponds and ditches drained into the Roseau through runoffs at four stations within the study area.

Densities of brook sticklebacks were recorded at numerous sites within each station. Collections were made using a small seine, and all fish collected were preserved and counted. The area of each seine haul was measured so that densities of sticklebacks could be estimated. Water velocity and temperature were recorded, and substrate, water depth, turbidity, and aquatic vegetation were described for each seine haul. The area seined for each collection was as homogeneous as possible with respect to the above variables.

Laboratory study — Laboratory experiments were conducted on a hypothesis to explain orientation of the fish during their upstream movements in the field study, namely, that they are positively rheotactic and select the warmest available water below 19–20 C.

Preferred temperatures of sticklebacks from the Roseau were determined before and just after spawning. Reproductive state was determined by secondary sexual characteristics (Baggerman 1957; Reisman and Cade 1967). Fish were held at temperatures similar to those at which they had been caught. Preferred temperatures were measured within 24 hr of capture in a horizontal temperature gradient, produced in a painted copper trough (100 × 10 × 5 cm). The trough was supported in styrofoam containers, so that water in the containers

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enveloped the ends and a portion of the sides. One container was warmed with a heater and the other was chilled with ice to produce a stable gradient of water temperatures (0–29.4 C) in the trough.

Precautions were taken to reduce the effect of factors other than temperature on distribution of sticklebacks in the trough. Light was reflected off a white ceiling so that intensity of illumination was similar along the gradient. A black cloth, with slits for viewing, separated the observer from the trough. Sticklebacks were tested singly. In preliminary experiments, distribution of sticklebacks in the trough was random when there was no temperature gradient (MacLean MS 1969).

A standard procedure was used to test each group of at least 10 fish. After the temperature gradient was established, the trough was drained and refilled to a depth of 3 cm with water at a temperature similar to that in which the group had been held after capture. One fish was placed in the trough, and after 6–8 min the gradient was reestablished. Thirty minutes after the fish was introduced, 10 observations were made of its position at 30-sec intervals. Water temperatures at the observed positions were then measured with a variable resistence thermometer. The fish was removed, the trough drained, and the procedure repeated until all fish had been tested.

An experiment was conducted in an artificial stream to determine the effects of temperature on movement of prespawning sticklebacks in current in light and in darkness. The stream, 9.15 m long and 16 cm wide, was based on a design used by Raleigh (1967). The stream bed consisted of gravel (2–20 mm diam) except that five pools without gravel were spaced at equal distances along the channel to provide areas of reduced current. Each pool could be sealed off with a wooden slat, so that numbers of fish in each section of the stream could be determined at any time. Water flowed at a surface velocity of 8 cm/sec over the gravel. Water depth was 2 cm over the gravel and 14.5 cm in the pools. Two pumps returned water from the downstream end of the channel to the upstream end. Five lamps, evenly spaced along the stream, reflected light off a white ceiling on to the stream.

Five groups of 20 sticklebacks were collected from the Roseau River on each of five consecutive days and tested within 24 hr at 7.2, 10.0, 15.6, 21.1, or 23.9 C (±0.15 C) Prior to testing, the group of fish was placed in the central pool midway along the length of the stream and contained there for 6 hr by slats. The slats were then removed, the pumps turned on, the fish exposed to current for 8 hr in darkness and the number in each section recorded. The fish were then returned to the central release pool, and exposed to current for 8 hr in light, and number in each section recorded.

Field study — Following breakup of river ice during early spring, brook sticklebacks moved upstream from overwintering areas into warm, shallow, meltwater ponds and ditches with low water velocity and reproduced there in May and early June. The fish evidently moved into the warmest water available below 19–20 C.

The most striking feature of the distribution of the fish during early spring was the concentration of large numbers in the temporary meltwater runoffs. Water in runoffs was often warmer than that of the river, especially during the day. In April and early May, sticklebacks were densest just inside the mouths of these runoffs, but only when runoff temperatures exceeded those of the river. Later, in May, sticklebacks moved upstream in runoffs into temporary ponds, where spawning occurred.

At station 1, a series of meltwater ponds drained through a shallow runoff into the river (Fig. 1). Water velocities in the ponds and runoff were 8 cm/sec or less. On April 7, 23, and May 5, sticklebacks were most abundant in the mouth of the runoff (sites 15–16), where water temperatures were warmer than those in the river nearby (Table 1). Sticklebacks were first observed in the meltwater ponds (sites 11–14) on May 14, and extensive seining the following day showed that the highest density was in the warmest pond, furthest upstream from the river (site 11).

At station 2, a shallow meltwater runoff emptied into a tributary of the Roseau. Water flowed into a pool at the head of the runoff from a culvert overhanging the pool, preventing fish from moving upstream. Water velocity in the runoff was 12 cm/sec or less. Again, as at station 1, a temperature gradient existed between the warm meltwater and the cool tributary of the Roseau. The densities (number/m²) of prespawning sticklebacks in the temperature gradient (C) were as follows:

	Pool	Runoff	Mouth of runoff
May 11 Temp	8.3	8.3–6.1	6.1
Density	0	0.7	0
May 23 Temp	16.7	16.7–15.0	15.0
Density	334.3	0.7	0
May 26 Temp	21.1	21.1–16.7	16.7
Density	449.2	36.1	0

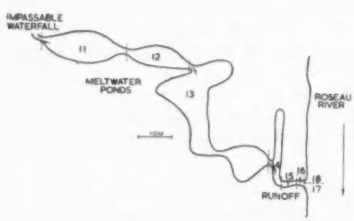

FIG. 1. Sites 11–18 at meltwater ponds and runoff draining into the Roseau River at station 1.

TABLE 1. Water temperatures and densities of brook sticklebacks (average number/m²) in several sites at station 1 during spring 1967.

Date	Site 11	12	13	14	15	16	17	18
Apr. 7								
Temp (*C*)					7.2	7.2–0	0	0
Density					119.4	250.7	0.6	0.2
Apr. 23								
Temp (*C*)					6.1	6.1–1.7	1.7	1.7
Density					32.1	0.2	0	1.7
May 5								
Temp (*C*)					14.4	14.4–5.6	5.6	5.6
Density					37.6	1.2	0	
May 15								
Temp (*C*) Max	26.7	23.8	21.0	18.2	18.2	17.8	17.0	17.0
Min	23.9	21.1	18.3	15.6	18.2	17.8	17.0	17.0
Density	40.8	6.2	1.1	0.1	2.7	0	0	

At station 3, a large meltwater runoff emptied into the Roseau. Ten meters upstream in the runoff, an impassable waterfall prevented further upstream movement of fishes. A temperature gradient existed between the warm waterfall and the cool river, and water velocities in the runoff were variable. Density of sticklebacks on April 7 appeared to be influenced by both water temperature and velocity. They were densest at low water velocities in the runoff mouth, where runoff and river water mixed:

	Runoff			
	Meters below waterfall			
	0–4	5–8	9	10(mouth)
Temp (*C*)	6.1	6.1	6.1–3.3	3.3
Water velocity (*cm/sec*)	122	30	92	0
Density (*sticklebacks/m²*)	30.1	61.1	1.2	5, 208

	2–4 m downstream from mouth of runoff
	Roseau R.
Temp (*C*)	2.2
Water velocity (*cm/sec*)	30
Density (*sticklebacks/m²*)	7.2

Movement into the runoff appeared to be restricted by water velocities greater than 90 cm/sec. On April 23 and May 5, river and runoff temperatures were identical, and no sticklebacks were captured.

At station 4, a large roadside ditch drained several temporary ponds into a large deep pond (Fig. 2). The ditches and temporary ponds were dry during the fall of 1967 and sticklebacks overwintered in the permanent pond. During the spring of 1968, meltwater drained from the temporary ponds to the permanent pond. On April 8, no temperature gradient was found along the runoff, and sticklebacks were not caught in the ditch, despite extensive seining. From April 18 to 30, a temperature gradient existed along the ditch, and schools of more than 20 fish were observed moving upstream in slow-flowing water. An upstream–downstream trap in the ditch revealed that movement of sticklebacks upstream increased in daylight and after a rise in water temperature, whereas movement downstream increased in darkness or when water temperature exceeded 19–20 C (MacLean MS 1969).

Observations of upstream movement of brook sticklebacks at the junction of the main ditch and a second ditch indicated that when water temperatures in the ditches differed, sticklebacks entered and moved up the warmest. However, if temperature in the warmest exceeded 19–20 C,

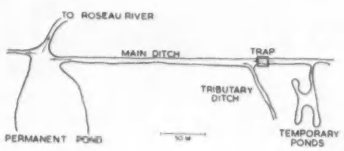

FIG. 2. Ponds and ditches draining into a tributary of the Roseau River at station 4.

sticklebacks selected the cooler ditch. When temperatures in both ditches exceeded 19–20 C, upstream movement ceased (MacLean MS 1969). Nests, and later fry, were found in the temporary ponds.

Laboratory study — In April, prespawning adults selected a range of temperatures from 14.9 to 20.2 C and averaging 17.3 C, considerably higher than the temperature of capture. In late May, postspawning fish selected a similar mean temperature, but the range and variation were greater, indicating a more precise response to temperature by adults just prior to reproduction.

Date	Temp (*C*) at capture	Preferred temp (*C*) Mean	Range	Coefficient of variation (%)
Apr. 30 (prespawning)	10.0	17.3	14.9–20.2	11.1
May 28 (postspawning)	14.4	16.3	8.9–25.6	26.4

In light in the artificial stream, greater numbers of prespawning adults moved upstream at 15.6 and 21.1 C than at either higher or lower temperatures (Table 2). But numbers moving downstream were similar at all temperatures tested. At extreme temperatures more fish tended to remain in the release pool. Ratios of the number of fish found upstream, downstream, and in the release pool at each temperature were compared in a 3×5 contingency table. The ratios differed significantly ($P < 0.05$), indicating that temperature influenced upstream movement of sticklebacks in light.

Net movement of fish in darkness was downstream at all temperatures, and movement was not significantly influenced by temperature.

To determine if illumination influenced the movement of sticklebacks in the artificial stream, tests at 15.6 and 21.1 C were combined and the results were tested in a 2×2 contingency table (direction of movement vs. illumination). Illumination was found to have a significant ($P < 0.01$) effect on the direction of movement of sticklebacks in current.

Discussion — During spring, adult brook sticklebacks in the Roseau River moved from deep cold water into shallow warm water, where they reproduced. Several authors have observed a similar movement by brook sticklebacks (Barker 1918; Evermann and Clark 1920; Winn 1960; Reisman and Cade 1967). Observations in the field and laboratory indicate that movement of prespawning adults was influenced by water temperature and current.

The ranges of temperatures preferred by prespawning brook sticklebacks in the laboratory gradient and the experimental stream (14.9–20.2 C and 15.6–21.1 C) are similar to that required by the species for successful reproduction. Reproduction activity occurs between 15 and 19 C, and outside this range nest building and courtship are seriously impaired (Winn 1960; Reisman and Cade 1967). Braekevelt and McMillan (1967) found that prolonged exposure to temperatures above 20–22 C caused cessation of spawning and atresia of mature oocytes. Lamsa (1963) reported a sudden downstream movement of brook sticklebacks in a small stream when water temperatures during spring exceeded 19 C. Reproductive activities require a narrower range of temperatures than most other activities, and response to temperature was less precise after reproduction.

In both field and laboratory, prespawning adults moved upstream in current, suggesting a positive rheotactic response. This tendency to move upstream was influenced by light intensity and water velocity as well as water temperature. Upstream

TABLE 2. Distribution of prespawning sticklebacks in an artificial stream (current velocity 8 cm/sec) at different water temperatures after 8 hr of either light or darkness. Fish moving upstream, moving downstream, and remaining in the central release pool were counted.

Temp (*C*)	Light			Darkness		
	No. upstream	No. in release pool	No. downstream	No. upstream	No. in release pool	No. downstream
7.2	7	6	7	2	1	17
10.0	7	6	7	2	0	18
15.6	13	2	5	7	1	12
21.1	12	1	7	8	0	12
23.9	6	8	6	3	0	15

movement was greater in light than in darkness. Harden Jones (1963) found that *Gasterosteus* uses visual cues to maintain position in current, and sticklebacks would have a greater chance of losing their position in darkness. Field observations suggested that prespawning brook sticklebacks prefer the slowest water velocities while moving upstream.

The hypothesis that a positive rheotactic response and a preference for water temperatures between 15 and 19 C orientates prespawning brook sticklebacks seems adequate to explain distribution and movements of the prespawning adults in the Roseau River. An alternative hypothesis is that homing might occur, but return of brook sticklebacks to their parental pond or ditch would be of limited selective value in the Roseau, as the same breeding sites are not present from year to year due to varying amounts of meltwater. Sticklebacks might, however, be attracted to a chemical component characteristic of the water discharged by runoffs. To detect this component, sticklebacks would have to use either olfactory or gustatory receptors, both of which are greatly reduced in *Gasterosteus* (Niauwenhuys 1959; Bannister 1965). Brook sticklebacks were found in runoffs only when runoff temperatures were higher than those of the river, suggesting that temperature, rather than a characteristic chemical component, guides adults into runoffs. This observation also argues against another hypothesis, that brook sticklebacks accumulate at runoffs to feed on drift from the productive meltwater areas.

The environmental requirements of brook sticklebacks for successful spawning include water temperatures between 15 and 19 C, vegetation for nest construction, and clear water for courtship displays (Winn 1960). These requirements are found during spring in meltwater ponds and ditches, but not in the main channel of the Roseau, which remains turbid, cool and fast flowing until mid-July, and where aquatic vegetation is scarce. Thus, a pattern of responses to temperature and current have evolved to guide brook sticklebacks to areas in the environment where chances for survival and reproduction are greatest.

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Temperature Requirements for Growth and Survival of Larval Ciscos (*Coregonus artedii*)

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MCCORMICK, J. H., B. R. JONES, AND R. F. SYRETT. 1971. Temperature requirements for growth and survival of larval ciscos (*Coregonus artedii*). J. Fish. Res. Bd. Canada 28: 924–927.

Temperatures above 13 and below 18 C are recommended as most suitable for sustained production of larval cisco, as indicated by instantaneous rates of growth, mortality, and net biomass gain in cisco reared at seven constant temperatures from 3 to 21 C. Growth rates increased in the temperature range from 3 through 18 C. Mortality rates were low from 3 through 13 C, but increased significantly between 13 and 18 C. Net rates of biomass gain were maximum at 13 through 18 C. The 24-hr median lethal temperature for ciscos acclimated to 3 C was calculated to be 19.8 C.

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THE cisco, *Coregonus artedii* Lesueur, is probably the most temperature-tolerant of the coregonids, but it is also one of the least temperature-tolerant fishes inhabiting inland lakes (Frey 1955). The success of a year-class of the cisco, as in many species, is believed to be established in the first year of life, the most critical periods being the embryo and larval stages (Kramer and Smith 1962; Franklin and Smith 1963; Toetz 1966). Thermal requirements of the embryo were reported recently by Colby and Brooke (1970), who determined optimum temperatures for successful incubation to lie between 1.5 and 8.5 C and 100% mortality at 12 C and higher. Edsall and Colby (1970) determined that the ultimate upper lethal temperature of juvenile ciscos (average length 48 mm) was 26 C.

The purpose of this note is to provide information on the thermal requirements of cisco larvae as shown by rates of growth, mortality, and net biomass gain at seven constant temperatures.

Materials and methods — Mature ciscos were collected in the fall of 1967 from Clearwater Lake, Cook County, Minnesota. The eggs were stripped and fertilized in the field, transported immediately to the laboratory, and incubated at 3 C.

Samples of 60 newly hatched larvae were randomly assigned to duplicate treatments (chambers A and B). At this time, two samples, one of 10 and the other of 11 fish, were also drawn from the pooled stock to determine initial weights. Subsequently subsamples of 10 fish each were drawn from the test lots at 14 and 28 days, except for errors in six subsamples in which the number of fish ranged from 7 to 11. The test lots were acclimated to nominal test temperatures of 3, 7, 10, 13, 16, 18, and 21 C by raising the temperature at a rate of 3 degrees C an hour.

The test system provided a continuous flow of water at approximately 250 ml/min at constant temperatures to duplicate test chambers (McCormick and Syrett MS 1970). The glass test chambers were 10 × 10 × 40.5 cm long. The water supply for all test chambers was carbon-filtered Duluth, Minnesota, city water with the following chemical characteristics, as determined from three samples at weekly intervals:

	Mean	Range
Total hardness (*mg/liter as $CaCO_3$*)	44	44–44
Total alkalinity (*mg/liter as $CaCO_3$*)	40	40–40
*p*H	7.7	7.6–7.7
Total residual chlorine (*mg/liter as chloramines*)	<0.004	≤0.001–0.004
Calcium (*mg/liter*)	13.8	13.8–13.8
Magnesium (*mg/liter*)	2.8	2.8–2.8
Dissolved oxygen (% *saturation*)[a]	96	90–100

[a]Samples were drawn from the outlet ends of test chambers.

Water chemistries were determined by the techniques outlined in American Public Health Association et al. (1965). Test temperatures (Table 1) were monitored with constant-recording telethermometers and were measured daily in each test chamber with a mercury thermometer with subdivisions of 0.1 degree C. Test temperatures were maintained within ±0.1 C (95% confidence limits) of the reported mean.

Larvae were fed *Artemia salina* nauplii ad libitum with the possible exception of weekends, when less

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TABLE 1. Instantaneous rates of growth, mortality, and net biomass gain of larval ciscos at constant temperatures, expressed as percent per day for the first 28 days after hatching. The values reported are weighted means of samples from two sampling dates from each of two replicates at each temperature.

Temp (*mean and range C*)	Growth (*wet wt*)	Mortality	Net biomass gain (*wet wt*)
3.4 (2.4–4.4)	2.43	0.44	1.99
7.0 (6.3–7.4)	5.40	0.26	5.14
9.8 (9.8–10.2)	8.08	0.46	7.62
12.8 (4.0–14.0)	9.98	0.70[a]	9.54[a]
15.6 (10.0–16.3)	12.50	1.39	11.11
18.1 (17.7–18.6)	13.46	3.84	9.62
20.8 (20.6–21.0)	15.62[b]	12.24[b]	3.38[b]
Tukey's honestly Significant difference	1.7	2.6	3.2
	$P = 0.05$	$P = 0.10$	$P = 0.10$

[a]Value for A replicate only.
[b]Value for first 14-day sampling period only.

than ad libitum feeding may have occurred. As Brown (1946) found that growth compensation soon followed a period of reduced ration when excess ration was again provided, the possibility of less than excess ration on weekends is considered inconsequential. Rearing units were cleaned and dead larvae counted and removed daily. Subsamples of larvae for weight measurements were removed from the test chambers before feeding on the morning of the sampling day and were held without food for about 6 additional hr before they were preserved. This fasting before preserving eliminated all or most of the weight that might be contributed by ingested but unassimilated food. The larvae were preserved in formalin–picric-trichloroacetic solution (Davenport 1960), blotted on paper toweling, and weighed to the nearest 0.1 mg.

Instantaneous rates of growth, mortality, and net biomass gain were calculated between sampling intervals on a percentage/day basis (Ricker 1958). Net biomass gain is the weight gained by all members of a test lot minus weight loss incurred through mortality within the lot. Analyses of treatment differences were made by one-way analysis of variance and Tukey's multiple range test (Steel and Torrie 1960). Disease in unit 13B resulted in excess mortality and deletion of mortality and net biomass gain data for that chamber, necessitating the use of missing plot procedure in analysis (Li 1964). Data obtained from 21 C test lots were not included in the analysis because of the high 1st-day mortality rate, which prevented projection with confidence of the 14-day data over the 28-day period used in all other comparisons.

Results and discussion — The instantaneous growth rate increased progressively as test temperatures increased from 3 to 18 C (Fig. 1). Increases in rates of growth were significant ($P < 0.05$) at each temperature increase from 3 to 16 C. The increase in growth rate between 13 and 18 C is statistically significant, but that between 16 and 18 C is not ($P < 0.05$, Table 1).

Instantaneous mortality rates were low from 3 through 13 C (Fig. 1). However, over the range from 13 to 18 C the rates became significantly higher ($P < 0.10$, Table 1). Mortalities reached 75% within the first 24 hr after being transferred from the 3-C incubation temperature to the 21-C test temperature. At 18 C, the next highest test temperature, 24 hr mortality rate was only 9.2%.

The 24-hr TL_{50} for fish acclimated to 3 C was 19.8 C. This value agrees well with that found by Edsall and Colby (1970), who found a lethal temperature of 19.75 C for this species when acclimated to 2 C. Further support for the validity of this laboratory-derived lethal temperature is given by the behavior of young-of-the-year ciscos, which have been found to leave the epilimnion for deeper water when the epilimnion temperature reaches 20 C (Fry 1937).

Net rates of biomass gain increased progressively from 2.0%/day at 3 C to 11.1%/day at 16 C

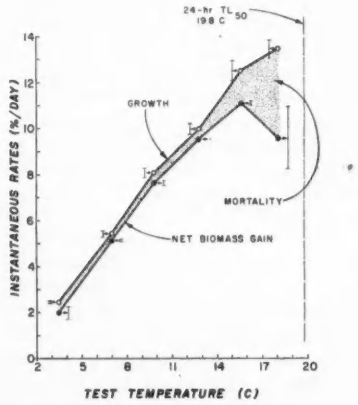

FIG. 1. Effects of temperature on mean instantaneous rates of growth, mortality, and net biomass gain of larval ciscos during their first 28 days after hatching. and the 24-hr TL_{50}. All plotted values are means of test chambers A and B except at 13 C, where only chamber A was used. The vertical lines set off from the means represent the ranges.

and declined to 9.6%/day at 18 C (Fig. 1). Differences in net rates of biomass gain are significant ($P < 0.10$) between the maximum at 16 C, and the lower rates at 10 C and cooler (Table 1). At 10 C and cooler, the low mortality rates fail to compensate for the lower rates of growth, but at 13–18 C the higher rates of mortality are compensated for by increased rates of growth. Increased net rate of biomass gain at higher temperatures may occur if larvae are acclimated to water warmer than 3 C, but 3 C is probably close to the lower end of the range of temperatures occurring naturally at hatching, particularly in larger lakes.

The thermal requirements of larval ciscos must be related to their specific habitats. Larval ciscos are more tolerant of high temperatures than are adults (Colby and Brooke 1969; Fry 1937) but bathymetric distribution of the larvae makes them particularly vulnerable to natural and artificial heating. Adults are considered deepwater fish, but they spawn in relatively shallow water. Larvae are found near shore in shallow protected areas and bays (Van Oosten 1929; Pritchard 1930). Larvae also are dependent on access to the surface to fill the gas bladder (Tait 1960).

The optimum temperature range for the larvae in a particular habitat may differ from that found in this study because of the effects of temperature on food requirements and efficiency of utilization of the food ingested for growth. The requirements for food intake are increased as temperature increases (Winberg 1956), and the efficiency of food conversion to growth is reduced (Gray 1929; McCormick MS 1960). In consequence, as food becomes limiting, the temperature for optimum growth is progressively lowered (Brett et al. 1969; T. A. Edsall personal communication).

The present study emphasizes the need to consider both mortality rates and growth rates in judging a suitable temperature for population growth, in this instance, a strong year-class. The importance of larval growth has been pointed out by Burrows and Combs (1968) who stated that maximum growth rates are desirable as evidenced by sea-run salmon, where it has been demonstrated that young of the same age but larger size survive at significantly higher rates.

Although net rate of biomass gain in this experiment did not increase statistically at temperatures above 13 C, the rates were high over the range of 13–18 C. It is suggested, therefore, that 13–18 C is the most suitable temperature range for the well-being of larval ciscos. The probability is high, however, that temperatures lower than 18 C are desirable when conditions of food supply, predation, competition, and water quality are less than optimal.

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Extension of the Known Range of the Masked Greenling, *Hexagrammos octogrammus*, to British Columbia

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Peden, A. E. 1971. Extension of the known range of the masked greenling, *Hexagrammos octogrammus*, to British Columbia. J. Fish. Res. Bd. Canada 28: 927–928.

Previously, the known geographic range of the masked greenling, *Hexagrammos octogrammus* (Pallas), extended north and west of Sitka, Alaska. The capture of four juvenile specimens from Stephens Island near Prince Rupert extends the known range by more than 200 miles and provides the first records of this species from British Columbia.

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Quast (1968) indicated that the southeastern limits of the known geographic range of the masked greenling, *Hexagrammos octogrammus* (Pallas), extended to near Sitka, Alaska. The known range of this species can now be extended more than 200 miles southeastward into the marine waters of British Columbia on the basis of four specimens (National Museums of Canada, Cat. No. NMC65-176) identified by the late W. Van Vliet. These pelagic stage juveniles, along with 15 similar juveniles of the whitespotted greenling (*H. stelleri*) and a juvenile kelp greenling (*H. decagrammus*) were seined at Qlawdzeet Anchorage, Stephens Island (54°12′N, 130°47′W) on June 12–13, 1965.

All of the specimens of *H. octogrammus* listed above have a long upper or first lateral line and a short fourth lateral line, characters that Wilimovsky (MS 1958) used to distinguish Alaskan specimens of *Hexagrammos*. Because lateral lines are often difficult to find on juvenile preserved specimens, the following counts were taken to facilitate identification:

Specimen no.	Standard length (*mm*)	No. spinous dorsal fin rays	No. soft dorsal fin rays	No. anal fin rays	No. of precaudal vertebra	No. caudal vertebra
1	43.0	19	23	23	18	33
2	42.4	19	25	24	19	34
3	44.9	19	24	25	–	–
4	39.0	19	24	24	19	33

The above dorsal, anal, and vertebral counts are within the ranges of those described by Quast (1964) for *H. octogrammus*. Most importantly, the 18 or 19 precaudal vertebra and 19 spinous dorsal fin rays recorded above for the specimens of *H. octogrammus* from British Columbia are outside the range of dorsal or vertebral counts of any other species of *Hexagrammos* that Quast

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recorded from North American waters. This confirms the identification of these juveniles as *H. octogrammus* and provides the basis for the first record of this species from British Columbia.

Acknowledgments — Mr C. G. Gruchy provided the radiographs from which vertebral counts were made in this report.

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Food of Two Cohabiting Tide-Pool Cottidae

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NAKAMURA, R. 1971. Food of two cohabiting tide-pool Cottidae. J. Fish. Res. Bd. Canada 28: 928–932.

The organisms found on seven dates in the stomachs of two cottids (*Oligocottus maculosus* and *O. snyderi*) cohabiting four rocky tide pools on the west coast of Vancouver Island were the same except for one minor item. Quantitatively, a number of differences were present. For the major food items, the interspecific differences in relative composition of the diets were greatest for amphipods and copepods. Lesser differences are seen for annelids, isopods, and chironomid larvae. However, these were more likely a reflection of differences in microhabitat and behavior rather than interspecific competition for food.

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THERE have been numerous comparative studies on the food habits of cohabiting species of closely related fish, especially those from fresh waters. These investigations have shown that often the diets of such cohabitants differ qualitatively (Fryer 1959; Nilsson 1955, 1958, 1960, 1965; Nikolskii 1963). This phenomenon has been regarded by these workers as the result of a mechanism whereby competition for food is reduced or eliminated.

The primary objective of this study was to determine whether such qualitative diet differences exist between two congeneric marine species that inhabit rocky tide pools. A second objective was to obtain information on food habits of sculpins (Cottidae), which are commonly the dominant fish species in tide pools. Except for the work of Mitchell (1953), Johnson (1954), and Sasaki and Hattori (1969), little is known of the trophic relations of this group of fish.

The two species selected for study were the tidepool sculpin (*Oligocottus maculosus* Girard) and the fluffy sculpin (*O. snyderi* Greeley). The former species has a wide intertidal distribution occupying pools at all levels of the intertidal. *Oligocottus snyderi* has a more restricted distribution, inhabiting only mid- and lower pools. In the areas of overlap, there is a potential for interspecific competition for food or "interactive segregation" (Nilsson 1955, 1958, 1960, 1965), or both.

Oligocottus maculosus and *O. snyderi* are morphologically very similar, the more obvious differences being the number of rows of cirri on the lateral surfaces of the body, male secondary sex characteristics, colors, and pigmentation patterns. Both are bottom-dwelling carnivores with similar spawning habits and life histories. *Oligocottus snyderi*, however, tends to be a more secretive species.

This research was conducted on the rocky intertidal zone of Port Renfrew near San Juan Harbor on the west coast of Vancouver Island, near the entrance to the Juan de Fuca Strait (48°32′N, 124°27′W). Direct exposure to the North Pacific

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Ocean makes this area subject to frequent intense wave action resulting from oceanic storms, which minimized field collections and observations especially during the winter and spring.

Methods — All fish used in this study were collected with a chemical fish toxicant. A total of seven collections were made in four different tide pools of midlevel from May 1966 to March 1967. Only stomach contents of individuals from pools in which both species occurred were used. The total lengths ranged from 34.2 to 91.3 mm for *O. maculosus* and from 34.3 to 89.3 mm for *O. snyderi*.

Immediately after collecting fish, 10% formalin was injected into the viscera to curtail digestive processes. The specimens were then placed in 10% formalin and later transferred to 40% isopropyl alcohol for permanent storage.

Results — Qualitatively, the food spectra of the two species were identical except for the occasional consumption of small fish by *O. maculosus* (Table 1). The foods common to the two species were amphipods, isopods, copepods, decapods, ostracods, caprellids, annelid worms, chironomid larva, and molluscs.

In spite of the qualitative similarities, a number of large differences are apparent (Table 1, 2). In terms of relative composition, amphipods, isopods, copepods, and annelids comprised the greater proportion of the diet of *O. snyderi*. These food items also contributed significantly to the diet of *O. maculosus*. However, copepods, which made up a relatively small part of the diet of *O. snyderi*, contributed numerically to a great extent to that of *O. maculosus*. Also, more chironomid larva were consumed by *O. maculosus*. Other food organisms such as decapods, ostracods, caprellids, molluscs, and fish were of minor importance since they each contributed less than 1.0% to the respective diets. Of the major food items, the greatest interspecific differences in relative composition of diets are apparent for amphipods and copepods.

When these data were analyzed in terms of frequency of occurrence of particular food items in all stomachs containing food, the food spectra of the two species were even more similar. Organisms most commonly eaten by *O. snyderi* were amphipods, annelids, isopods, and copepods. These same items, in addition to chironomid larva, were also the most frequently occurring foods in the diet of *O. maculosus*. Minor food items such as ostracods, decapods, caprellids, and molluscs were observed more frequently in stomachs of *O. maculosus*.

Of the major foods common to both species, the greatest differences in frequency of occurrence of specific items in the respective diets were observed for annelids, copepods, and chironomids. Of these, the latter two were eaten more often by *O. maculosus*.

Discussion and conclusions — The results show that cohabiting *O. snyderi* and *O. maculosus* feed essentially on the same food organisms. Since this does not conform to the separation of food spectra between competing species observed by Nikolskii (1963) and Nilsson (1955, 1958, 1960, 1965), it is suggested here that food is not a limiting factor and that competition between these two species is nonexistent in the diverse and complex intertidal pool environment. Mitchell (1953), in his study of food habits and possible competition between tide-pool fishes of Southern California, reached a similar conclusion. According to Mitchell, there is "little competition for food" between the carnivorous tide-pool fishes.

The apparent absence of competition between the two cottids in the tide pools of Port Renfrew is in accord with field observations made by the writer and a number of bottom and epiphytic samples that indicate high, though variable, levels of abundance of littoral amphipods, isopods, and annelids throughout the year. Nilsson (1955, 1958, 1960, 1965) attributed similarities in diets of cohabiting trout and char in northern Swedish lakes to an abundance of food and consequent breakdown of niche barriers.

The quantitative interspecific differences in diets can be explained on the basis of numerous field observations on the microhabitats and behavior of the two species of fish by the writer and R. Boyd (unpublished data). Mitchell (1953) also cited behavior and habitat differences in accounting for quantitative diet differences between *Gibbonsia elegans* and *G. metzi*, two tide-pool blennoids that display microdistribution relations identical to those of *O. maculosus* and *O. snyderi*.

The greater consumption of copepods and chironomids by *O. maculosus* is probably directly related to common occurrence of this species in the more open areas of tide pools where these organisms are most apt to occur.

The higher proportions and greater frequency of amphipods, annelids, and isopods in the diet of *O. snyderi* can be related to the more secretive nature of this species and its close association with the substrate and surf-grass (*Phyllospadix scouleri*) and coralline algae (R. Boyd unpublished data, and Nakamura MS 1970). Annelid worms are very abundant in the tangled mats of *Phyllospadix* roots. Amphipods are usually observed in close

TABLE 1. Relative percent composition of diets of *O. maculosus* and *O. snyderi* (upper row = *O. maculosus*, lower row = *O. snyderi*).

Date	Pool	*n*	Amphipoda	Isopoda	Copepoda	Annelids	Insecta	Caprella	Ostracoda	Decapoda	Mollusca	Pisces
						1967						
Feb. 3	C	16	59.5	4.1	14.9	2.7	12.2	0.0	2.7	1.4	2.7	0.0
		1	57.1	14.3	0.0	28.6	0.0	0.0	0.0	0.0	0.0	0.0
Mar. 15	D	17	60.3	12.3	1.4	15.1	4.1	0.0	0.0	6.8	0.0	0.0
		4	36.4	9.1	9.1	18.2	0.0	0.0	9.1	18.2	0.0	0.0
Mar. 15	B	17	34.3	12.4	36.2	10.5	1.9	0.0	1.0	1.9	0.0	0.0
		1	85.7	0.0	0.0	0.0	14.3	0.0	0.0	0.0	0.0	0.0
May 17	B	21	5.6	2.4	86.6	1.2	3.0	0.0	0.2	0.2	0.6	0.2
		3	14.1	74.6	7.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0
						1966						
June 5	A	16	24.4	1.3	63.8	1.8	6.3	0.0	1.3	0.3	0.3	0.7
		21	57.5	18.3	9.2	7.8	3.3	0.2	0.7	0.7	2.6	0.0
Oct. 1	B	29	12.6	2.0	77.5	2.5	2.3	0.0	0.0	2.5	0.0	0.7
		8	62.5	12.5	28.1	12.5	0.0	12.5	0.0	0.0	0.0	0.0
Dec. 12	A	4	82.5	10.7	1.9	1.9	0.0	1.9	0.0	0.9	0.0	0.0
		69	78.1	5.1	1.7	14.6	0.0	0.2	0.0	0.4	0.4	0.0
Pools combined		117	23.6	3.5	61.4	4.3	4.3	0.1	0.7	1.0	0.4	0.4
		107	70.0	11.8	3.6	12.2	0.6	0.5	0.2	0.6	0.6	0.0

TABLE 2. Percent frequency of occurrence of foods in the stomachs of *O. maculosus* and *O. snyderi* (upper row = *O. maculosus*, lower row = *O. snyderi*).

Date	Pool	*n*	Amphipoda	Isopoda	Copepoda	Annelids	Insecta	Caprella	Ostracoda	Decapoda	Mollusca	Pisces
						1967						
Feb. 3	C	16	57.1	57.1	14.3	14.3	14.3	0.0	14.3	7.1	14.3	0.0
		1	100.0	100.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Mar. 15	D	17	76.4	41.2	5.9	52.9	11.8	0.0	0.0	24.4	0.0	0.0
		4	50.0	25.0	25.0	50.0	0.0	0.0	25.0	50.0	0.0	0.0
Mar. 15	B	17	70.9	47.1	35.0	47.1	11.8	0.0	5.9	11.8	0.0	0.0
		1	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0
						1966						
May 17	B	21	65.0	45.0	75.0	25.0	40.0	0.0	5.0	5.0	10.0	5.0
		3	100.0	100.0	33.0	0.0	33.0	0.0	0.0	0.0	0.0	0.0
June 5	A	16	93.8	31.3	75.0	43.8	81.3	0.0	25.0	12.5	12.5	25.0
		21	90.4	47.6	19.0	57.1	19.0	0.0	4.8	4.8	19.0	0.0
Oct. 1	B	29	65.5	24.1	27.6	20.7	6.9	0.0	0.0	20.7	0.0	6.9
		8	87.5	25.0	37.5	37.5	0.0	12.5	0.0	0.0	0.0	0.0
Dec. 12	A	4	100.0	50.0	50.0	50.0	0.0	25.0	0.0	25.0	0.0	0.0
		69	91.3	31.9	14.5	81.5	0.0	1.4	0.0	4.3	4.3	0.0
Pools combined		117	71.8	31.6	39.3	33.3	25.6	1.7	6.8	15.4	3.4	6.0
		107	89.6	36.4	17.8	69.2	6.5	1.9	1.9	5.6	2.8	0.0

association with masses of coralline algae and surf-grass and in the cracks and crevices in tide-pool walls. Cirolanid isopods are in greatest abundance under rocks and debris and pentidotheid isopods are most abundant on *Phyllospadix* leaves. Because *O. snyderi* does not venture far from its hiding places, it is more likely to encounter and be restricted to these food organisms than those that occur in the more open areas of the tide pools.

Oligocottus maculosus, being less secretive and less restricted in its movements, will frequently venture into the thick maze of surf-grass and under rocks in addition to the more open areas within tide pools. As a result, it encounters a greater variety of food organisms, which is reflected in higher frequencies of occurrence of more food items in its diet.

Since the data were analyzed on the basis of numbers rather than volume or weight, the importance of minute food organisms such as copepods was overestimated whereas that of larger food such as amphipods and annelids was underestimated. Thus, *O. maculosus*, though frequently consuming greater numbers of copepods than amphipods or annelids, probably derives less net value from the copepods when considering the biases mentioned above. It appears to the writer that amphipods are the most important food source for both species of fish.

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